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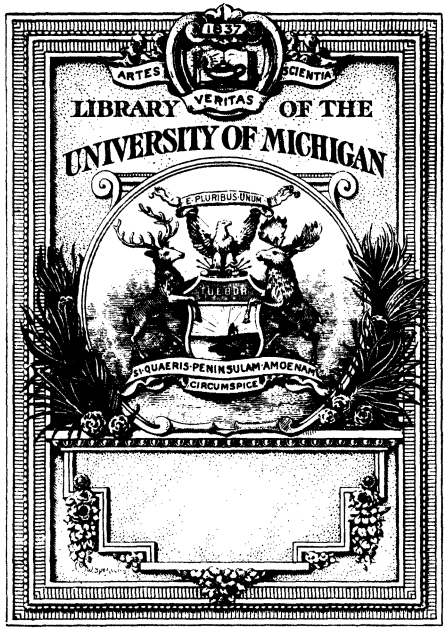

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No. 18.—OCTOBER, 1904

DEPARTMENT OF THE INTERIOR
BUREAU OF GOVERNMENT LABORATORIES
BIOLOGICAL LABORATORY

PART I

AMEBAS: THEIR CULTIVATION AND ETIOLOGIC SIGNIFICANCE

BY

W. E. MUSGRAVE, M. D., AND MOSES T. CLEGG

PART II

TREATMENT OF INTESTINAL AMEBIASIS (AMEBIC DYSENTERY) IN THE TROPICS

BY

W. E. MUSGRAVE, M. D.

MANILA
BUREAU OF PUBLIC PRINTING
1904

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1904

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LETTERS OF TRANSMITTAL.

DEPARTMENT OF THE INTERIOR,
BUREAU OF GOVERNMENT LABORATORIES,
OFFICE OF THE SUPERINTENDENT OF LABORATORIES,
Manila, P. I., August 10, 1904.

SIR: I have the honor to transmit herewith a report on (1) Amebas: Their Cultivation and Etiologic Significance, by W. E. Musgrave, M. D., and Moses T. Clegg; and (2) Treatment of Intestinal Amebiasis (Amebic Dysentery) in the Tropics, by W. E. Musgrave, M. D.

I am, very respectfully,

PAUL C. FREER,
Superintendent Government Laboratories.

Hon. DEAN C. WORCESTER,
Secretary of the Interior, Manila, P. I.

DEPARTMENT OF THE INTERIOR,
BUREAU OF GOVERNMENT LABORATORIES,
BIOLOGICAL LABORATORY,
Manila, P. I., August 4, 1904.

SIR: I have the honor to submit herewith and to recommend for publication reports on (1) Amebas: Their Cultivation and Etiologic Significance, by Dr. W. E. Musgrave, Pathologist Biological Laboratory, and Mr. Moses T. Clegg, Assistant Bacteriologist; and (2) Treatment of Intestinal Amebiasis (Amebic Dysentery) in the Tropics, by Dr. W. E. Musgrave.

Very respectfully,

RICHARD P. STRONG,
Director Biological Laboratory.

Dr. PAUL C. FREER,
Superintendent Government Laboratories.

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PART I.

AMEBAS: THEIR CULTIVATION AND ETIOLOGIC SIGNIFICANCE.

By Dr. W. E. MUSGRAVE and Mr. MOSES T. CLEGG.

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SYNOPSIS.

I. Introduction.
II. Cultivation.
- Review of literature.
- Various media (including our own).
- Cultural technique.
  - Bacterial symbiosis.
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    - Cultivation of pure species of amebas.
    - Cultivation of pure species of amebas with pure cultures of bacteria.
    - Selectiveness of amebas for special bacteria which may be symbiotic with them.
    - Special symbiotic bacteria.
- Distribution of amebas and sources from which they may be obtained by culture.
  - (*a*) Externally.
  - (*b*) Animals other than man.
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- Cultural characteristics (general).
  - (*a*) Morphologic.
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  - (*d*) Reaction to physical conditions.
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- Cultivation of special amebas.
  - (*a*) Ameba 11524.
  - (*b*) Ameba 11147.
  - (*c*) Ameba "water."
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  - (*g*) Ameba 34354.
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  - (*i*) Ameba 12485.
  - (*j*) Ameba 12483.
  - (*k*) Ameba "H."
  - (*l*) Ameba "L."
  - (*m*) Ameba "C."
  - (*n*) Ameba "urine."

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III. Etiologic significance.
- (*a*) Amebas are harmless commensals.
- (*b*) They intensify or alter the lesions already present.
- (*c*) There are pathogenic and nonpathogenic amebas.
- (*d*) All amebas are or may become pathogenic.
- (*e*) Animal experiments.

IV. Concluding remarks.

V. References to literature.

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PART I.

AMEBAS: THEIR CULTIVATION AND ETIOLOGIC SIGNIFICANCE.

By W. E. MUSGRAVE, M. D., and MOSES T. CLEGG.

I. INTRODUCTION.

The cultivation of amebas,[1] while a subject of the greatest importance, has never excited a very general interest, especially in America; and, although there is an extended literature on the subject, it is scattered over a long period of time and unfortunately some of the earlier writings deal with work not performed with proper care.

This early and unsatisfactory work accounts partly for the small amount of attention which the more careful articles of recent times have received, and materially adds to the responsibility of those who now take up the subject. Some of the confusion and doubt which exist in regard to cultivation and etiology is also due to the fact that a number of those who have grown amebas have, although insufficient data were at hand, devoted much time to a discussion of the biology and classification of the parasites.

The controversy relative to the etiologic significance of these parasites in human disease has been active almost since the time of the very important observations of Lösch, and, to judge from much of the recent literature, is still far from being decided. Any attempts at the solution of these problems must take this doubt into consideration, and every detail of the subject must therefore be made clear.

[1]The authors have followed Dorland's Dictionary in spelling.

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In this first paper we have purposely avoided a detailed discussion of the life cycle, the classification, and other strictly biologic questions regarding amebas, but instead have taken up their cultivation from various sources, including thte dysenteric intestine, and have proved their etiologic role by animal experiment with cultures of diverse origin, and have shown that at least some amebas are pathogenic. Certain of these pathogenic ones are found in places which indicate methods for the prevention of the infection, a consideration which is of great importance to us in the Philippine Islands.

The term "amebiasis," which we have introduced, is in line with modern nomenclature and is comparable in its application with uncinariasis, trypanosomiasis, filariasis, etc.; and indicates an infection with amebas. It is the only term thus far suggested which covers all grades of the infection and is readily adapted to the various locations in which such infection may be found. We need not here enter into a discussion of the defects of other names which have from time to time been advanced. It is almost universally admitted that none of them are entirely satisfactory and that many of them are inappropriate.

The general arrangement of the subject-matter has been indicated above, and it is given in this somewhat unusual manner because it meets the questions in the order in which they have appeared to us.

II. CULTIVATION.

Desultory attempts at the cultivation of amebas were probably made many years ago.

Aurbach (1856) asserts that he grew these organisms by exposing water containing a small piece of animal tendon to sunlight, but the more interesting and important observations are of a later date and follow Lösch's valuable work of 1875.

Cunningham (1879) cultivated amebas in boiled solutions of cow's dung and other substances. Grassi (1882) repeated and enlarged on Cunningham's experiments. Both of these authors believed that they had succeeded in growing *Amœba coli.*

Kartulis (1885) failed to grow *Amœba coli* in media composed of fluid blood serum or liquid gelatin. In 1890, with media composed of sterilized alkaline solutions of rabbit and pigeon dung, he believed he had been successful. His best media (1891), however, were either solutions of ordinary bouillon or sterilized alkaline decoctions of hay.

Kovacs (1892) repeated the methods of previous writers, but failed to cultivate dysenteric amebas by the use of any of them.

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Ogata (1893) asserted that he had obtained pure cultures of protozoa by a unique method which depended for its results upon a principle now generally known as geotropism. He used as a medium a 2.5 per cent solution of grape sugar in sterilized tap water. After growth had been established he separated the protozoa by the use of a small glass tube, which was nearly filled with the medium and was placed in a vertical position with its lower end in the culture. By the action of the negative geotropism on the part of the protozoa they wandered upward into the small tube, and after various lengths of time Ogata obtained lengths, by sealing it off and removing sections, which contained only one variety of the protozoa, which could then be transferred to other media. He succeeded in transplanting some of his protozoa in gelatin media.

Vivaldi (1893–94) maintained that he had grown *Amœba coli* in sterilized straw decoction, and had produced diarrhea in cats by infection with these cultures.

Kruse and Pasquale (1894) repeated and criticized the previous work on the cultivation of amebas and concluded that *Amœba coli* had not theretofore been grown. By following the technique of others they obtained amebas, but neither by any of the previous methods nor by more elaborate ones were they able to obtain *Amœba coli.*

Miller (1894) cultivated, in the presence of other microörganisms, amebas in a variety of fluid media. One-fifth per cent milk in hydrant water, dilute bouillon, and hay infusions were considered the most satisfactory. Dilution of the media and a large exposed surface were thought requisite.

Celli and Fiocca (1894) in a preliminary report stated that they had grown amebas free from other microörganisms, with the exception of bacteria, on solid media. In a complete report which appeared in 1895 they described their methods in detail. They were successful in growing amebas in several media, including alkaline potatoes, ascitic fluid, and egg albumen, their most satisfactory one, however, being 5 per cent *Fucus crispus* in bouillon or water which was strongly alkaline.

Picardi, Perroncito, and Bosso (1894) claimed successful cultivation on agar media.

Fijardo (1896) cultivated in straw decoction media amebas from dysenteric stools, but did not consider them to be *Amœba coli.* Beyerinck in the same year grew amebas together with bacteria or torulae on several solid media. One species developed well on ordinary nutrient agar and gelatin.

Schardinger (1896–97) cultivated, on solid media, amebas which had been obtained from stools and other sources. The medium which he recommended was made from agar, hay, water, and calcium hydrate.

Peyrot and Roger (1896) cultivated amebas on media composed of straw infusions, but did not regard the organisms as *Amœba coli.*

Frosch (1897) grew amebas on solid media. He thought special media not to be so necessary to growth as satisfactory living bacteria. His *Amœba nitrophilia* grew well on ordinary agar provided the proper bacteria were present. He experimented with several media, among others, the *Fucus* of Celli and Fiocca, the agar of Beyerinck, the hay infusion of Schardinger, aromatic gelatin, potatoes, charred turnips, and beets. None

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of these were satisfactory. He obtained good results with asparagin and glycogen solutions; but the simplest medium and the one most suitable for his amebas was composed of agar one-half per cent, bouillon 10 per cent, and tap water 90 per cent. On this the parasites always grew well when in the presence of bacteria.

Casagrandi and Barbagallo (1897) used both fluid and solid media, and claimed success with both. They also obtained positive results with the sterile white of egg, to which sodium bicarbonate had been added.

Jensen (1898) asserts that he successfully cultivated amebas from the intestine and other sources, on a solid medium, which was prepared from barley sprouts fifteen days old, which were cooked in water, filtered, rendered alkaline, mixed with agar, and autoclaved.

Tsujitani (1898) grew amebas on solid media prepared from straw, jigartina prolifera, agar, and sodium carbonate. He also used with success the media recommended by Frosch.

Zaubitzer (1900) repeated the work of Celli and Fiocca as well as that of several other experimenters, and, although by these methods he succeeded in obtaining amebas from a number of substances, the results were not satisfactory. He recommended as more suitable media, solutions of somatose, either in liquid or as a 1 to 2 per cent somatose agar made alkaline.

Mouton (1902) was successful in the cultivation of amebas from garden earth. His medium was practically that used by Frosch and his results in general were confirmatory of that author's work. Mouton succeeded in securing his amebas in pure culture with one species of bacteria, and isolated a diastatic ferment therefrom.

Gottstein (1903) succeeded in cultivating amebas on solid media. He used Dietjen's blood platelet medium, on which a small variety of ameba grew very well; but another would not grow even when somatose, as recommended by Zaubitzer, was added. Besides this he used various combinations which had been previously recommended. The somatose-agar of Zaubitzer, in the proportions advised by that author, was not so successful in his hands; but when somatose was used in smaller amounts in combination with sodium chloride and agar the medium thus obtained proved very satisfactory. His most sucessful results, however, were secured with the dilute bouillon agar of Frosch.

Our own work on the cultivation of amebas has been going on for some years. We have from time to time repeated the work of the principal experimenters, but usually, when following their methods, with negative or unsatisfactory results, so far as the cultivation of amebas from the intestine is concerned.

Particular attention has been given to the *Fucus crispus* medium of Celli and Fiocca, but we have been unable to confirm their results. In following their directions regarding the preparation and alkalinity of the medium, we have been able to obtain only a semi-solid product, which was neither transparent nor colorless—prop-

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erties so essential for plate work. We were able to grow straw amebas on it, but have never succeeded with those from the human intestine.

The somatose-agar advised by Zaubitzer has been somewhat more satisfactory. But, as stated by Gottstein and others who have repeated his work, we have found that to obtain favorable results, a much smaller proportion of the somatose than has been recommended should be used. Better results have been obtained with the bouillon-agar medium recommended by Froch and used by Mouton, Gottstein, and others. It has been especially satisfactory when symbiotic bacteria have been present.

It would be useless to describe all the various media which we have employed. Amebas from water, hay, soil, etc., may be grown indefinitely on a large variety of them, but for the cultivation of those which have passed through the alimentary canal of man and other animals the choice is not so great.

As a stock medium we have come to use the following preparation:

Agar	20.000
Sodium chloride	.300 to .500
Extract of beef	.300 to .500

Prepare in the same manner as ordinary nutrient agar. The finished product is most universally satisfactory when 1 per cent alkaline to phenolphthalein.[1]

Variations from this stock medium, consisting in the use of a still smaller amount of sodium chloride and beef extract, or in leaving out the salt entirely, will sometimes be found advantageous, especially when the amebas are growing in company with a very luxuriant saprophytic bacterium. On the other hand, a very small

[1] In order to obtain a final reaction of 1 per cent alkaline to phenolphthalein it will usually be found necessary to start with an initial alkalinity of 1.5 per cent. After autoclaving the medium in a flask, filtering the precipitate, distributing in tubes, and autoclaving again, the final product will be clear and will have an alkalinity of about 1 per cent.

Wherry (Bulletin No. 19 of this Bureau) has recently discussed the precipitation of acid albumins in alkaline media and its effect upon both the reaction and the nourishing properties of the finished product before and after the removal of these substances. We have found that our media are more satisfactory when these precipitated albumins are filtered off, although by this method the nutritive value for bacteria is somewhat diminished.

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quantity of peptone sometimes improves it, when a very delicately growing bacteria is being used. When this is desirable the simplest way to prepare the medium is to use from 5 to 10 per cent of ordinary laboratory bouillon and 2 per cent agar in water.

Technique (diagnostic).—Before continuing the discussion of the results we obtained by cultivating the protozoa a description of methods for the study of amebas, separated either from the feces or from cultures, should include a consideration of fresh preparations and of the various staining reactions. The course to be followed in determining the presence of amebas in the feces has been so universally described in text-books and the literature in general that repetition here is not essential.

There are, however, two points in this connection to which we wish to call attention. The first is the error of the general recommendation to select from a stool a mass of mucus as most advantageous for microscopic examination, because it is supposed to contain a larger number of organisms than material from other sources. *To get the best results the patient should always be given a saline cathartic and the examination should be made from the fluid portion of the stool.* Salts not only make the examination easier and the results more reliable, because of the fluid stools which they produce, but in addition they give rise to a larger number of parasites in the specimen, owing to the fact that in such a procedure the entire colon is washed and amebas which may be propagating in it in various locations are thus flushed out; whereas in the examination of a natural passage, even though the patient has diarrhea parasites from the cecum may not be passed.

In the second place, the diagnosis of amebas should never be made unless they are in a motile state. Errors are often committed in this way, even by the experienced. Ordinarily one may be reasonably certain of the diagnosis of typical resting or encysted forms; yet even here mistakes are occasionally made. In the interest of the patient, further examinations should be insisted on after the use of a saline cathartic.

In addition to the examination of fresh specimens, many other methods of diagnosis have from time to time been suggested. Probably none of them have any especial advantage, if indeed they are as certain as the use of the fresh material. A few of them will be noted.

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Zorn recommends the following method for preparing stained and permanent preparations:

A few cubic centimeters of feces are mixed with three or four volumes of a solution consisting of fifteen parts of 1 per cent solution of chromic acid and three parts of 1 per cent solution of osmic acid. The mixture is shaken thoroughly and after ten minutes is centrifuged. The sediment is then mixed with five volumes of a 25 per cent solution of Beale's carmine, allowed to stand for one-half hour and again centrifuged. The sediment is then washed in a weak, rose-colored solution of the same carmine and mounted in glycerin, or first dehydrated and then mounted in balsam.

Doflein recommends for fixing either of two solutions:

I.—

Saturated aqueous solution $HgCl_2$	100
Alcohol	50
Acetic acid	5

II.—

Picric acid	2
Alcohol	50
Acetic acid	5

According to this author, the protozoa may be fixed as a thin film spread on a slide, or they may be handled in bulk, embedded in paraffin and treated as sections.

For staining, the following dyes may be used: Borax carmin, Grenacher's hematoxylin and eosin, gentian violet, saffranin, Heidenhain's iron hematoxylin, or Romanowsky's methylene blue and eosin.

A number of writers have spoken of staining *in vivo* by neutral red and a few other substances.

By running a dilute solution of neutral red under a cover-glass preparation from a culture, beautiful pictures may be obtained. (See figs. 24 and 25.) When young motile amebas are present they take up the stain without interfering with their motility for considerable periods of time, and while there is not a great deal of differentiation, the bright color makes it possible to follow the parasites closely and to observe the character of their movements. This stain will also give a fair picture of resting amebas, but for encysted ones it is useless.

For staining permanent preparations of amebas from culture the most satisfactory method we have yet used is Wright's modification of that of Romanowsky, and the technique is the same as the

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one recommended for blood films. By applying this method to fresh cover-glass impressions from plate cultures, the amebas are often fixed in various phases of motion and the general structure is very well shown. It does not as a rule give a satisfactory nuclear picture. (See figs. 7, 8, 9, and 10.)

Craig has recently, by a modification of this method (not yet published), succeeded in staining amebas from the stools in such a manner as to bring out all the details. He maintains that he has been able to demonstrate by this means that *Amœba coli* multiplies by sporulation, a belief which also has been held by several other writers since Cunningham, some of whom, including Schaudin, have claimed its demonstration.

The known methods of staining have not been so satisfactory in our hands as could be desired, and any improvements in this particular line will, no doubt, yield valuable results when applied to amebas in culture.

Technique (cultural).—The media and some of the methods to be employed have already been discussed; but attention must also be given the methods of securing bacterial symbiosis and of isolation as well as to other points. Throughout these experiments the technique used to prevent contamination, etc., has been the same as that employed in bacteriologic work.

In obtaining cultures from water and from most other external sources, a large sample of 100 to 500 cubic centimeters of water, or of an aqueous solution or suspension of other substances, is collected in a cotton-plugged, sterile flask, to which is added 0.5 to 1 cubic centimeter of ordinary 1 per cent alkaline buillon to each 100 cubic centimeters of sample. The flask is then set aside for from twenty-four to seventy-two hours, when an examination will usually reveal amebas on the surface of the liquid. A loop of this culture is then spread over the surface of a Petri dish upon which the melted medium recommended in another place has been poured and allowed to harden. In the course of from six to forty-eight hours the plate should be examined under a low power of the microscope, as is done in the study of bacterial colonies; the amebas can then readily be seen and followed. Finally, the culture may be transferred frcm plate to plate or inoculated on ordinary slants of the same medium.

As a rule, with cultures from such external sources little or no care will be necessary in regard to satisfactory symbiotic organ-

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isms, as amebas are less selective under such circumstances and suitable bacteria are carried over in sufficient numbers to nourish the parasites. In isolating them from stools, however, much more care is necessary in this respect. We have never been able to obtain amebas from this source by first inoculation into fluid media of any kind. In plates prepared according to the preceding recommendations we have occasionally succeeded in securing growths simply by lightly smearing the surface with material selected from feces containing amebas. Success in such cases probably means that satisfactory organisms have been carried over in sufficient numbers and have been so distributed as to give the parasites nourishment. Positive results by this means, however, are not constant, but fortunately there is opportunity for improvement.

Owing to the selectiveness of amebas for certain bacteria, and particularly of amebas from the intestine on their first transplant, in order to grow them with more constancy, the surface of several plates should be smeared with pure cultures of various kinds of bacteria and then inoculated with the intestinal contents. It will be found that amebas will develop upon one or more of these plates while showing no evidence of doing so on any of the others. There are no means of determining beforehand what organisms will be satisfactory to an artificially uncultivated ameba, but by using a variety, comprising from six to twelve species, the percentage of positive results can be very much increased. By the use of twelve different selected bacteria we have succeeded in obtaining growth in 30 per cent of one series of cases, where the control inoculation made on the same medium without bacteria showed only 2 per cent of positive results.

Where it is desired to cultivate amebas from any particular intestine, and the means already mentioned have been tried but have failed, there is still a possibility of obtaining growth by first making cultures on ordinary agar plate from the same intestine and using the bacteria which develop as symbiotic ones when the material containing the amebas is added. By this method we have grown amebas in three out of five cases, where all other methods had failed.

Amebas containing red blood cells probably do not reproduce, and it is possible that there are other conditions of environment or phases of the life cycle which play an inhibiting part in certain cases. In one instance we have succeeded in obtaining a sat-

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isfactory growth by first causing the amebas to encyst through exposure for twelve hours in the ice box, when all of our cultures from the specimen, which had not been treated in this way, failed to develop. In other instances we have grown the protozoa from stools in which they could not be positively demonstrated by careful microscopic examination.

The first plates taken from the stool or from the intestinal ulcers must be watched frequently and carefully under the microscope, and as soon as it is found that amebas have developed (which they do after a period varying from twenty-four hours to four or even five days) transplants must be made. If this is not done promptly the parasites are liable to die, and no further growth will take place. When the amebas have once become accustomed to the artificial media, however, less trouble will usually be found.[1]

Symbiosis with bacteria.—Almost since the time when the first attempts at the cultivation of amebas were made, bacteria have been found associated with them, and much time has been devoted to the study of methods looking to the separation of the two classes of organisms.

Baumgarten (1890) and others before and since that time have pointed out the probability of coöperative action between amebas and bacteria in the production of dysentery; and this was followed by suggestions that the association of the animal parasites and bacteria was not an accidental condition, but a true symbiosis. More extensive and satisfactory experiments in cultivation, based upon the supposition that this symbiosis is necessary to the life of the protozoa, have been developed in recent years.

Janowski (1897), in one of the best reviews of this subject which we have seen, says, that "it is not improbable that the symbiotic organisms may determine the pathogenicity of the amebas themselves, and that the two may be associated outside and carried together into the alimentary canal, or that the former (bacteria) may already be in the intestine."

Views less far-reaching but similar in trend have been held by many of the most careful workers both before and since Janowski's observations were made. Prominent among these Beyerinck, Schardinger, Frosch, Mouton, Zaubitzer, Gottstein, and several others may be mentioned.

The existing data upon the extent and importance of this question will naturally be brought out in the discussion of attempts to obtain pure cultures of amebas.

Pure cultures.—Many methods have been employed for this purpose, but few if any have ever been successful; and even in those

[1] In keeping stock cultures, transplants have been found successful with some amebas after six and one-half months, while with others they have been found to die unless much more frequently transferred.

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cases where the parasite was supposed to have been isolated, its satisfactory development never took place when transplants were made.

Kartulis (1890) thought that, on a medium consisting of a straw decoction, he had obtained a pure culture of amebas from an abscess of the liver culturally free from bacteria.

Ogata (1893) maintained that he had secured protozoa in pure culture. His method consisted essentially in taking advantage of the principle now quite generally known as negative geotropism, which is a movement peculiar to a number of the protozoa.

Celli and Fiocca (1895) attempted to eliminate the bacteria by various methods, such as exposure of the mixed cultures to 55° and 60° C. for various lengths of time, filtration, placing disinfectants in the media, and inoculation into animals, thus endeavering to secure pure cultures. But when at length, after great pains, they succeeded in obtaining amebas free from bacteria, the former did not reproduce on transplants.

Casagrandi and Barbagallo (1897) concluded that it was impossible to separate the amebas from the bacteria in the ordinary media. By the use of the *Fucus crispus* medium of Celli and Fiocca they secured cultures free from bacteria. They also succeeded in isolating amebas from a culture made with yeast. These writers revert to the original idea that the association of the protozoa and bacteria is an accidental one and that the life and propagation of the former are not dependent upon the presence of the latter.

Frosch (1897) took old encysted cultures of his *Amœba nitrophilia* and treated them at room temperature for from seventy-two to seventy-four hours with a 20 per cent solution of sodium hydroxide. This process he proved by cultural experiments destroyed nonspore-bearing bacteria but not the ameba cysts.

The latter grew when transplanted to media inoculated with suitable bacteria, but without, they always failed to develop. It then occurred to Frosch that certain products of the bacterial life might fill the requirements and that in this manner it might be possible to obtain pure cultures. With this object in view he used various filtrates from growing cultures and from digested bacteria, but all of his experiments proved negative. He therefore concluded that his *Amœba nitrophilia* was not a saprophyte, but was dependent for life upon nourishment found only in living micro-örganisms.

Tsujitani (1898) obtained amebas in pure culture together with cholera vibrios by methods which will be mentioned presently, and then destroyed the latter by heating at 60° C. for forty minutes. He thereupon planted the encysted amebas in sterile media, where, although they became active, they did not multiply; but multiplication did take place when they were transferred to pure cultures, or to killed cultures of certain bacteria. We may therefore say that he had a *pure* culture of amebas. He arrived at the same result by another method. Sterile silk thread was dipped into a culture containing amebas and cholera bacilli and afterwards dried in the dessicator. By this process the cholera bacilli were killed, but the

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amebas remained as cysts and were again placed on media with bacteria, where they developed in pure culture. Transplants on sterile media, however, were not satisfactory. Tsujitani concluded that when amebic cysts free from other organisms are inoculated on sterile media, they develop to the vegetative stage but do not further multiply, but that when living bacteria are added a luxuriant growth occurs. Living bacteria, therefore, form the most suitable food for these protozoa, but dead bacteria may also nourish them, as proved by the following experiment. Tsujitani took old cultures of a favorable symbiotic organism, heated them for one hour at 60° C. and then plated them to see that all the organisms were dead. These dead cultures were then inoculated with ameba cysts and development occurred, though not so luxuriantly as with living bacteria.

Zaubitzer (1901) by means of a 20 per cent solution of sodium hydroxide as recommended by Frosch, killed the bacteria occurring in encysted cultures of amebas on straw infusions. These cysts grew when transplanted to media inoculated with a suitable living bacterium, without the presence of which it was impossible to obtain a growth upon the same or any other medium. Killed cultures of bacteria were also employed, but without success. He therefore concluded that living bacteria are essential to the life of the parasites.

Mouton (1902) failed to secure amebas in pure culture and concluded that bacteria were necessary to the propagation and nourishment of the parasites.

Several others have attempted to grow amebas in pure culture by mechanical and other means, but the methods above described are the most important. With the possible exception of Tsujitani's work, which has not been confirmed, no one as yet has succeeded in obtaining satisfactory pure cultures of amebas which continue to develop on successive transplants.

Following the methods of Frosch, after making controls, three-month-old encysted cultures of three of our amebas ("11147," "11524," and "water") were washed with a 20 per cent solution of sodium hydroxide and then allowed to stand for seventy-two hours. Transplants were then made on sterile media and on media inoculated with organisms favorable to the growth of the amebas. Cysts were readily demonstrated in the alkaline solution at the time of transplanting as well as on the inoculated cultures at the end of twenty-four hours; but no growth had occurred. It was thought possible that in making transplants a sufficient amount of the strong alkali might have been carried over to prevent growth; and in order to obviate this contingency a second set of cultures was made after carefully neutralizing the alkali with hydrochloric acid, but without altering the results. We also varied the time of exposure to the alkali, and the strength of the solution; but we have never succeeded by this method in obtaining any development of amebas on transplants after bacteria have been destroyed. The

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principal work done by others along this line has also been repeated by us.

Certain old cysts of amebas have been found to be more resistant to heat than are some of the bacteria, and after much work we have succeeded in destroying the latter without killing the amebas, as evidenced by the fact that the former grew on being transplanted to media which were simultaneously inoculated with another satisfactory symbiotic organism. The absence of the first organism from these transplants was determined by plate culture. Transplants of any of our amebas to bacteria-free media did not induce growth.[1]

We have employed many other methods, including those of other workers, as well as a considerable variety which have not been previously described, but always with negative or doubtful results.

The only environment in which amebas are found to be apparently free from bacteria is that furnished by certain culturally bacteria-free liver abscesses. If these abscesses in reality do not contain other organisms, it probably proves that there are substances which are capable of nourishing amebas under certain conditions and which are not microörganisms. What is already known would naturally lead us to look upon such substances as ferments or enzymes.[2]

As we shall see below, amebas combined with various symbiotic bacteria in pure culture, if injected directly into the liver, cause abscess, in which both organisms may be found on exploration. We have recently made attempts to utilize this fact in a practical way. For this purpose, for instance, a monkey was first immunized against cholera spirilla, and after immunity had reached a high degree, a pure mixed culture of amebas and *Spr. choleræ* of the same stem as that used to give immunity was injected directly into the liver. The animal was killed on the fifth day after injection, but no abscess was found at necropsy, though the inoculation wound in the liver could be plainly seen. Smears from the liver did not show either amebas or bacteria. The control monkey, which received the same treatment without immunization, had an abscess at necropsy which contained amebas and *Spr. choleræ*.

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[1] The method mentioned above of drying cultures on silk threads has not been repeated by us up to the present time.

[2] Richet has shown that the normal liver contains a proteolytic enzyme in not insignificant quantities; and with this fact in view we now are experimenting with hopes of securing cultures free from living bacteria.

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Various other attempts have been made to reproduce artificially a condition similar to that present in bacteria-free liver abscesses with the hope of securing satisfactory pure cultures; but they have invariably been unsuccessful.[1]

It will be seen therefore that satisfactory pure cultures of amebas have not yet been obtained. The work of nearly all recent authors, as well as our own, *seems* to point to the impossibility of such a procedure, because a satisfactory symbiotic living microörganism is indispensable for the nourishment of these protozoa. However, failures to accomplish this purpose, so serious from a practical standpoint, do not prevent us, as we shall show in this paper and subsequent ones, from proving the etiologic significance of these parasites, by taking advantage of well-regulated symbiosis with bacteria.

The cultivation of a single species of ameba.—Having demonstrated that amebas from various sources will grow on a variety of media in the presence of other microörganisms, and having indicated the most satisfactory medium thus far known for this purpose, and finally having shown that amebas fail to propagate by any known methods in the absence of other microörganisms, or at least of organic life in some form, and that there are good reasons for believing such organisms or life really necessary for the growth of these protozoa, we are in a position to take up the next logical step in our work, namely, the study of methods for obtaining in culture a single species of ameba. For this purpose, in order to be accurate it will be necessary to start transplants from a single individual. Various methods, mostly mechanical, have been employed to this end.

Celli and Fiocca (1895), so far as we have been able to ascertain, are the first who called attention to this necessity; they obtained their results

[1] We have destroyed the bacteria in pure mixed cultures of amebas and *Spr. choleræ*, by producing Pfeiffer's phenomenon in the abdominal cavity of a guinea pig. Amebas present in the fluid withdrawn after this operation are always encysted or destroyed, and no growth has ever been obtained on sterile media when the reaction was complete. On one occasion, however, we have been able to obtain a growth on media which were inoculated with another satisfactory symbiotic bacterium; and future experiments may determine this to be a useful method within certain limits for transferring the parasites from one bacterium to another. When old encysted cultures are used, it is probably the best way so far devised for obtaining amebas free from bacteria.

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mostly by the use of a mechanical finger. Others following them have used methods largely dependent for their success upon the same general principle.

Beyerinck and others maintain that they were successful in accomplishing the same purpose by varying the character of the media. This method, however, while it might allow of a separation of two species, does not prove either of them to be pure.

Ogata's method of obtaining pure cultures in capillary tubes might be of advantage, the requisite being to find one of the small sections which contains but a single ameba.

However, when plate cultures are properly made, so that a very scattering growth is obtained with isolated amebas (as may often be seen) at a considerable distance from one another, the mechanical process is not a difficult one.

The following procedure has given us greater satisfaction than any other: Select a plate culture on which the parasites are well distributed, and after removing the cover, place the plate with the open side up on the stage of the microscope. By searching the edges of the growth with a Zeiss AA objective, places will be found where the amebas are some distance apart (1 to 3 fields). After locating a satisfactory parasite, which should be one on the surface of the medium, as practically all of them are, and having determined that there are no others in the field, either on the surface or at a depth, swing a clean and perfectly dry DD lens in place and gently lower it until the entire surface is in contact with the medium. Raise the lens quickly, swing in the AA objective, and determine whether the ameba is still present or has been picked up by the DD objective. If it has been picked up, which after some practice may be done two or three times out of five, the lens to which the ameba adheres is removed and, by gently rubbing its surface over that of a plate containing the hardened medium, the organism may be transferred. In this manner a pure culture, so far as amebas are concerned, may be obtained from the multiplication of a single parasite. The possibility of picking up more than a single individual by this method may be offered as a criticism, but with attention to the details which have been given, as well as to those which will suggest themselves to the worker, this method is certainly less open to criticism than the one involving the use of mechanical fingers or any of the other ones previously described. That only one ameba has been carried over by this method may still further be verified by examining with an AA objective the closed inverted plate on which it has been inoculated.

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Another useful result of a careful application of this method is the aid it gives in obtaining pure cultures of amebas and of a single bacterium. The lens, of course, picks up the bacteria from a small field immediately surrounding the amebas; and as such isolated amebas are often surrounded by one kind of bacterium only, with the aid of a careful bacteriologic technique the pure cultures desired may sometimes be obtained in this manner. Methods of cleaning the lens and of obviating the entrance of air organisms readily suggest themselves. We have used the preceding method in most cases and have found it satisfactory. It would be possible to obtain the desired results more easily and with greater constancy by means of Unna's bacterial harpoon or a specially constructed lens, with a short adjustable focus and a cup-shaped extremity, like the marking arrangement which has been suggested for locating special fields in permanent preparations.

Cultivation of a single species of ameba with pure cultures of bacteria.—Having secured cultures of amebas of a single species, we are prepared to discuss the bacteria and methods of securing satisfactory symbiosis. A number of authors have reported the isolation of amebas with a single variety of bacteria; but apparently, very few have extended their work to include the isolation of a single species of the protozoa with a pure culture of a single kind of bacteria, the "pure mixed culture" of Frosch.

Beyerinck (1896), by allowing certain microörganisms to develop on plates, then inoculating the mixed cultures of amebas and bacteria at one point and watching the rapid development of the amebas, was able at times, to secure transplants which contained the desired bacteria and amebas only. By the same method he also isolated them with pure cultures of yeast.

The spread of amebas on the surface of media already inoculated with satisfactory organisms has been utilized by several others for the purpose of isolating the former with a single species of bacterium. With a slight difference in the mode of execution, this was one of the means employed by Tsujitani, Gottstein, Schardinger, Mouton, and others.

Some of the above-described methods for obtaining pure cultures, while failing in the specific object sought, were successful in isolating amebas in conjunction with a single organism. These procedures include the destruction of the bacteria in encysted cultures by heat, chemicals, and other substances, without eliminating the ameba cysts, which grow when transplanted to satisfactory media which have been simultaneously or previously inoculated with a suitable microörganism in pure culture.

In accordance with this principle, Zaubitzer, having satisfied himself that in contradistinction to bacteria, encysted amebas are not destroyed by 20 per cent sodium hydroxide, used this fact as a means of obtaining amebas

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in culture with a single organism. After treating the encysted cultures with the soda solution, transplants were made to satisfactory media, which were also inoculated with pure cultures of the organisms desired. He obtained a satisfactory growth of amebas with *Spr. cholerœ*, *B. typhosus*, *B. coli*, and other organisms.

Mouton (1902) secured amebas with one variety of bacteria by inoculating mixed cultures in plates which had been previously prepared by introducing the desired bacteria in lines radiating from the center. The amebas followed these lines more rapidly than did their associated bacteria, and were sometimes found with only the desired organisms. He also accomplished the same results by distributing colonies of the desired organism on plates of media, inoculating the latter with amebas which, as they wandered from one to the other of the bacterial colonies, lost the original organisms and eventually could be found pure with the desired bacteria.

Gottstein (1903), after inoculating the surface of plates with pure cultures of bacteria, brought upon the center a culture of amebas. These organisms then spread outwards until they reached the margins, and from this location it was possible to secure a pure culture with a single bacterium.

A satisfactory method of cultivating a single species of amebas with pure cultures of bacteria is, of course, absolutely necessary in order to accomplish reliable results. In repeating the work of others, we have found most of their methods tedious and uncertain. After many attempts the following satisfactory and quite simple routine has been developed, and it is now in daily use in our work. It is based on the principles maintained by Beyerinck, Mouton, and others, although simpler and more certain in its results.

The sterile ameba medium is melted and poured into ordinary Petri dishes, the usual precautions being taken. The dishes are then allowed to cool and the medium to become thoroughly hardened. With a platinum loop several rings of pure culture of the organism with which it is desired to grow the ameba are made on the surface of the hardened agar, and a small smear inoculation of the mixed culture of the amebas is placed in the middle of the smaller or central bacterial ring. (See figs. 1 and 2.)

If the necessary precautions have been taken, most amebas, as they multiply, will quite generally spread rapidly over the plate, and in passing through the rings of growing bacteria they will lose the organisms with which they started and take up those forming the rings. In from twenty-four to seventy-two hours the protozoa will have passed one or more of the rings, and from such locations they may be taken for transplanting. It sometimes happens that they appear on the first plate in pure cultures with the desired

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organism, but more generally one or more transplants to the same medium are necessary before this end is reached. The further inoculations are made with amebas obtained from outside the largest ring on the next preceding culture.

This method is simple in execution, and the whole process may be watched under the microscope by inverting the plate and using a low power, following the process used in studying colonies of bacteria. With a Zeiss objective AA and ocular 4 the wanderings of the amebas and even their multiplication can be kept under observation.

The ring-shaped smear of bacteria has several advantages over one covering the entire surface of the plate. In the first place, amebas develop more rapidly by its use, and, secondly, they lose the original organisms much more readily than when moving constantly over a bacterial substratum.

Another feature which commends this method is the readiness with which it lends itself to a determination of the symbiotic value of a given organism. If for any reason it is not satisfactory to the amebas, they will not mix with or cross the bacterial rings. In some instances, where the organism is particularly unfavorable, the amebas, after wandering up to the inner margin of the first ring, encyst, and no further progress is made. On the other hand, where the antipathy is less marked, the progress is simply delayed until the bacteria carried over in inoculating the amebas have mixed with or crossed the ring, whereupon the amebas follow them.

When amebas have been isolated and grown in pure culture with a satisfactory symbiotic organism, it is sometimes difficult to transfer them to another. This is best overcome by first cultivating the protozoa for a short time on mixed cultures of the two organisms and then isolating them with the desired one by the use of the method described. Even by this means success is often doubtful and sometimes impossible of attainment, probably because the organisms with which the amebas are already associated are much more satisfactory than are those with which it is desired to have them continue.

Another difficulty is found in transferring the amebas from a very profusely and rapidly growing organism to one which is more delicate. Fortunately, but few grow profusely on the medium recommended, but where such a difficulty arises, a medium in which the amount of nutriment is still further restricted will prove

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useful. This hindrance can further be overcome by allowing the rings to grow for from twenty-four to forty-eight hours after inoculation before placing the amebic culture in the center.[1]

The number of bacteria to be carried over by the inoculation of the amebas can always be greatly limited by selecting on the margin of the growth of a previous plate culture the place from which to make the transplant.

Selectiveness of amebas for special bacteria which are symbiotic with them.—Amebas show a selective action for certain bacteria; this is true whether the culture is pure or mixed. This fact has been noticed by several of the more recent writers on cultivating these protozoa, including Beyerinck, Frosch, Tsujitani, Zaubitzer, Mouton, Gottstein, and others, all of whom have advanced evidence to this effect. When we add to these observations the facts which have been brought out by our own work, we are justified in concluding, at least tentatively, not only that living bacteria are necessary to the life of amebas, both in the animal economy and under other conditions, but also that there is a natural selectiveness on the part of the parasites for certain species of bacteria.

This selectiveness is particularly marked in amebas from the human intestine and other parts of the animal economy, and is equally true whether they have been brought to these places by natural means or by experiment. This is shown by the difficulty with which amebas from such sources can be cultivated, success seeming to depend largely on placing the proper organism with them in sufficient numbers. When taken from these surroundings they often develop only on plates which have been treated with a particular culture. This holds true even when the bacteria which are used are cultivated from the intestine of the case which harbored the amebas. However, after the latter have been grown for some time on artificial media, they become less selective and may finally be made to develop in symbiosis with any one of a large variety of microörganisms.

[1]There is one difficulty sometimes encountered which it is impossible to overcome. Sometimes there are obtained in cultures certain very small amebas which do not move rapidly enough on the media and so reach the resting stage too early to allow of their separation from the bacteria. We have one such culture in our collection. It grows well on transplants, but we have so far entirely failed to secure it in pure culture with any organism.

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Amebas, whether found in the intestine or outside the body, are practically always associated with various kinds of bacteria; but it does not necessarily follow that all of these play a part in the metabolism of the parasites. This is indicated by the microscopic examination of amebas taken from stools and other places, when they often show bacteria embodied in their protoplasm; but usually there is no indication that more that one kind is present in any young, healthy, growing individual, although a great variety may exist in its environment.

These conclusions are still further corroborated by methods of culture. If plate cultures are made from a substance in which amebas are multiplying, and all the various bacteria are isolated in pure culture and transplanted to our media, it will be found that the amebas will multiply profusely in some, will grow very indifferently in others, while in certain ones no growth whatever will occur. If amebas from other sources are inoculated into control cultures of these organisms, similar results will be obtained, excepting that the organism producing a satisfactory symbiosis and therefore a satisfactory culture of the second amebas, may be one entirely different, morphologically, culturally, and even in pathogenic action, from the ones suited to the amebas from the first source. *In a word, amebas in a natural environment are selective; but this property may be overcome to a certain extent on artificial media, and perhaps also in ways which are more natural. On the other hand, this phenomenon is reversible, for, by passing the amebas through the animal economy, the selectiveness may be increased.*

One of the most important applications of these results, when more is known of the cultural characteristics of the amebas, may lie in the fact that a knowledge of this will enable us to classify amebas and also to obtain them in pure species. Amebas which are alike, but taken from different natural environment, may be found to grow in symbiosis with identical organisms and may reject others which are also identical, while, on the other hand, other varieties may choose different bacteria for symbiosis and satisfactory growth, and, thus, a biologic difference may be established. However, unfortunately, the ease with which amebas may be made to change their habits, and, hence, the almost utter impossibility of showing their previous pleasure in this respect, throws considerable doubt upon the hope of any success in the direction suggested.

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If, as has been shown, it is only with great difficulty that an ameba from an animal source can be grown, although it may be made to decrease its selectiveness by successive transplants on artificial media and may subsequently develop with any one of a variety of organisms, it becomes important to determine whether this process may be reversed. Repeated observations have, as stated above, proved that it may be, and that in so doing another element, which is not so easy of explanation, is encountered, namely, an acquired resistance to culture, apparently due to causes other than the lack of a satisfactory bacterial environment or symbiotic relationship.

It is easy to separate an ameba from Manila tap water which at first will grow with a large variety of organisms. If it is isolated with one variety and encysted cultures are obtained and injected, either subcutaneously or into the liver of an animal, abscess usually results, and the amebas from this abscess may again be grown with cultures of the introduced symbiotic organism and sometimes with other organisms. However, if the contents of this abscess or even cultures from it are transferred to another animal, it will be found more diffcult to obtain growth from this second one; and this difficulty will increase as successive animals are used, until it becomes as great or greater than that attendant upon growing amebas obtained from the dysenteric intestine of man. We have repeatedly failed to obtain a growth in the second and third animals, although the amebas were microscopically present in the abscess contents and we were sure that the original symbiotic organism was being used.

These facts make us reasonably certain that another element has entered into consideration in the nutrition of amebas, and one which becomes most markedly manifest when the liver is used as the seat of the abscess. In a measure we have here conditions analogous to those present in liver abscesses in the human body, for from these also we have not succeeded in cultivating amebas in three cases with which we have had the opportunity of experimenting. A quite plausible explanation of this phenomenon is tentatively advanced by us, namely, that by these successive inoculations we have increased the selectiveness of the parasite to a point where the bacterial symbiosis is replaced by something else. In searching for the probable nature of the substance selected, enzymes or ferments

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suggested themselves, some of which are known to be peculiar to the liver.

These facts render it possible that living bacteria are not really necessary to the life of amebas under all conditions. The trend of our work at the present time is corroborative of the foregoing results, and this further investigation will be embodied in another report, together with a discussion of the biology of the parasites.

There is still another question of importance in regard to this selectiveness and its variability, the answering of which is of the utmost importance from several points of view, namely, does the varying symbiosis, in addition to increasing the difficulties of cultivation, influence in any way the pathogenic nature of the parasite? For example, is an ameba in symbiosis with *Spr. cholerœ* more or less pathogenic than one after a long symbiosis with a harmless saprophyte? At the present time we are only able to state that we have produced experimental dysentery in monkeys with the same kind of amebas which at one time were in symbiosis with *Spr. cholerœ,* and at another with an absolutely nonpathogenic saprophyte, with no appreciable differences in the results; and furthermore, neither at the time when the dysenteric symptoms developed nor at necropsy were we able to obtain by culture the bacteria introduced with the protozoa. This would tend to support the view originally suggested by Janowski, namely, that it is not the nature of the first symbiosis but that of the one formed with the bacteria already in the intestine, which determines the pathogenicity, if indeed, the nature of the symbiosis is at all a factor in this question.

Whatever influences such habits may have on the pathogenesis, all the work which has been done points to the fact that it *is* the symbiosis formed *after* the introduction of the parasite into the intestinal canal which insures the propagation of the amebas there; and there is a great temptation to explain some of the negative results which have been obtained in experiments by the supposition that in the intestine of man or animals symbiotic bacteria satisfactory to the entering ameba were not present at the time of its introduction.

Having seen that such intestinal symbioses are formed in experimental animals, after feeding "pure mixed cultures" of amebas, and that it is just as difficult to reclaim the amebas from such intestines as it is from the human dysenteric ones, and finally that

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the organism most likely or satisfactory for such cultural symbiosis is not necessarially the one introduced, but one of many which may be obtained from the intestine itself by culture, we are brought back to a proposition already mentioned, namely, that it may not always be the bacteria themselves which are necessary for culture or pathogenicity, but it may possibly be some ferment or bacterial product which produces this change.

The following table indicates the selectiveness of three of our amebas after growing for several months on artificial media:

Various stock cultures of bacteria	Water.	11147.	11524.
17682	A.	B.	B.
17681	A.	B.	B.
11483c	A.	B.	A.
12004b	A.	X.	A.
9650d	A.	X.	A.
11604c	A.	X.	B.
9650b	A.	B.	B.
11604e	A.	X.	A.
11939e	B.	B.	A.
11483e	A.	B.	A.
9650e	A.	A.	A.
431′	B.	X.	X.
17683	A.	B.	A.
11939c	A.	B.	B.
17680	A.	B.	B.
11523b	A.	B.	A.
11939d	A.	X.	A.
11939f	A.	B.	A.

A = Good growth. B = Poor growth. X = No growth.

11147 and 11524 were amebas grown from dysenteric stools and were at the time of isolation very selective, being obtained with cultures of only one bacterium. Ameba "water," on the other hand, was isolated from the city water supply of Manila and showed very little selectiveness. Later, after being fed to monkeys, it became very much more so and was as difficult to reclaim from animal sources as any of those from the dysenteric intestine.

Special symbiotic bacteria.—The few observers who have grown amebas with a single microörganism have also found a variety of bacteria giving satisfactory symbiosis with their protozoa.

Beyerinck grew amebas with pure cultures of yeast as well as with a number of bacteria, including acetic bacteria and *B. coli.* Frosch was most successful with a nonsporebearing bacillus obtained from garden earth.

Tsujitani obtained successful results with *Spr. cholerœ, B. typhosus, B. coli, B. flourescens, liq., B. flourescens non-liq., Staphylococcus p. aureus, B. pyocyoneus, B. rubra,* and three varieties of bacilli from hay infusions. He failed to do so, however, with a bacillus from a liver abscess as well as with yeast and fungi.

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Zaubitzer's most successful organisms were nonpathogenic ones. Mouton was successful with *Spr. cholerœ, B. coli, Vib. metschnikoffi, Staph. p. aureus, B. anthracis, B. mallei,* and a yeast. Gottstein had the best results with bacteria obtained from garden earth.

Our own work has convinced us that the bacteria which under certain conditions produce satisfactory symbiosis include quite a large variety both of the pathogenic and of the nonpathogenic ones. Naturally, we have paid particular attention to the bacilli of the colon group, and it is with this class that we have had our most frequent successes in producing from the stools primary cultures which show the usual variations of the group. We have found that two organisms which are apparently identical culturally give different results as symbiotics for all amebas from a given source.

The first one with which we were successful was—

Bacillus 9650b.—Plates were made from stool 9650, and many colonies of all varieties of organisms contained therein were transferred to our media, and after three days all these cultures were inoculated from stool 11147 (dysentery). After forty-eight hours an examination showed that cultures made from colony *b* had many amebas, while those from the other ones were negative. This led to a more careful study of Bacillus 9650b. It appears to belong to the colon group, and morphologically and culturally does not appear to differ from others of the group. It is a rather short rod, slowly motile. It sometimes shows polar staining, while again it may be coccoid. It ferments glucose and lactose with the production of acid and gas. Litmus milk is quickly turned acid and coagulated by it.

Another organism which has proved very useful to us is *Bacillus 12935.* It is a yellow pigment-producing saprophyte, which may frequently be isolated from the city water supply or from plates exposed to the air. It is an organism which grows profusely, is motile, and measures about 0.5 μ by 1.5 to 2 μ. In numerous animal experiments we have most frequently used this bacillus as a bacterium capable of symbiosis with amebas, because of the ease with which it may be recognized and reclaimed and because it is nonpathogenic for our animals as well as for man.

Bacillus violaceus Manilæ (Woolley) was isolated by Dr. P. G. Woolley, Director of the Serum Laboratory, from the lungs of carabaos and is described in Bulletin No. 15, Bureau of Government Laboratories. This bacillus has been very useful because of the great antipathy which some of our amebas have shown for it. Two of our cultures of amebas encysted when first placed with this organism, and apparently no further multiplication occurred. However, by gradually, during a period of two months, increasing the proportion of this bacillus we have succeeded in obtaining a satisfactory culture with no other organisms present. But even under these circumstances this culture required frequent transplants. On the other hand, we have amebas which from the first are able to exist symbiotically with this bacillus.

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We have successfully used *Spr. cholerœ* and several other varieties of vibrios, which have been isolated from various substances during the cholera epidemic in these Islands. Other bacteria which have acted more or less satisfactorily with some one or more of our amebas are *Staph. p. aureus, B. typhosus,* and many organisms not identified, from the air, water, liver abscess, and the normal and dysenteric intestine.

Distribution of amebas and sources from which they may be obtained by culture.—It is almost universally true that those who have worked on this subject at all seriously have succeeded in cultivating amebas from a great number of sources outside the human body. According to reports coming from all parts of the world, they have been isolated from soil (both surface and deep, 2 meters), prairie and mountains (1500 meters), marshes, slime, stagnant water, thermal springs; river, lake, and sea water; air, dust, dried grass, hay, fruits (both sound and decayed), including acid, decaying grapes, and many other substances.

In the majority of instances, particularly among the more recent workers, all cultivated amebas have been considered to belong to the so-called nonpathogenic type, and have usually been dismissed with this supposition without sufficient effort to determine their pathogenic significance.

From the nature of amebiasis and for other reasons, we are justified in believing that water and other substances are transmitters of the disease, and therefore must at times contain the infecting agent. With this belief we are justified in paying particular attention to the amebas from these sources; and since it has been proved, as we shall presently show, that some of these are dysentery producers, their extracorporeal distribution becomes of the greatest importance. We, as well as other authors, have demonstrated them in a large variety of materials; of this class we shall discuss in detail only those obtained from two important sources, namely, water and uncooked vegetables.

Amebas are found in large numbers in almost all af the surface waters of the Philippine Islands, and may almost constantly be cultivated therefrom without difficulty. This becomes all the more interesting when we consider the city water supply of Manila.

Amebas have been grown from every one of one hundred samples of water taken from hydrants in different parts of the city during the past six months, and with three of these cultures we have been

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able to produce dysentery in monkeys. (Our culture "water" is from this source; it will be discussed in detail presently.) We have had no difficulty in cultivating amebas from the washings of vegetables, from a number of fruits, and from various other kinds of organic matter. Another of our cultures, "lettuce," was obtained from the fourth washing in distilled water of lettuce grown on the Government experimental farm in the city of Manila. Many surface soils in this country have been found to contain amebas in large numbers. We are now working with a number of cultures from soil taken at various distances from the surface.

In general, it may be said that the whole of the surface flora of the Philippine Islands carries a large number of these parasites. Some of which, at least, belong to the class which produces disease in human beings.

Distribution of amebas in animals other than man.—These protozoa undoubtedly have a wide distribution in the intestinal canal of many animals. They have been reported as present in the digestive tract of frogs, chickens, pigeons, lambs, calves, rabbits, dogs, horses, monkeys, and are probably existent in many other species.

Blanc found what he considered to be amebas in the lungs of a sheep. He says that the protozoa were larger than *Ameba coli*, had a single pseudopod, and produced in the lungs nodules resembling those seen in verminous pneumonia.

Generally no significance is given to the presence of amebas in the digestive tube of animals; they are thought by most authorities to be harmless. There are, however, a few observations which tend to show that this is not always the case, even when the amebas are ingested by natural means. Kartulis found them in the intestines of dogs which suffered from dysentery in Egypt. Cats have been reported to have the disease, and we have occasionally seen a spontaneous case in monkeys.

The question whether the passage of amebas through some of the larger animals increases their danger to man is one offering inducements for future work. It is just now particularly interesting to Manila because of the location and management of the new water supply. The present water sheds are inhabited by both human beings and animals; the new one will harbor only animals. The water from the present as well as the proposed new sheds contains large numbers of amebas. No practical means, on a large scale, has as yet been devised by which these protozoa may be removed from the water.

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Distribution of amebas in man.—Amebas in several pathologic conditions have been found in man, both by culture and by microscopic examination. The more common sources, such as the intestine, the liver, etc., need not be mentioned here.

Miura found amebas in ascitic fluid from a woman suffering with abdominal tumor. They were present also in the bloody, mucous stools.

Celli and Fiocca cultivated amebas from the larynx in a case of tubercular laryngitis, ten times from the lungs in tuberculosis, six times in cases of pulmonitis, in fifteen cases of bronchial catarrh, three out of fifteen times from the female urinary tract, and once from the stomach of an infant. Amebas have been reported in scrapings of tartar from the teeth.

Kartulis isolated these microörganisms from a necrotic bone in the lower jaw. Flexner found them in an abscess located on the floor of the mouth and in a gangrenous surgical wound in a case of liver abscess.

Leyden and Schaudin encountered ameboid bodies in aspirated ascitic fluid in two cases of abdominal tumor. Baelz found them in the bladder and vagina of a young woman who later died of tuberculosis of the lungs and genitalia and who had hemorrhagic cystitis.

Jurgens repeatedly observed amebas in the urine of a patient, 58 years of age, suffering with tumor of the bladder. They were found by Wijnhoff in the urine of four patients.

Posner discovered amebas, some of which contained red blood cells, in the bloody urine of a man who had never been out of Berlin. This patient was suffering from a disease which, with recurrent attacks, had lasted for a period of a year. It was Posner's belief that the amebas had reached the pelves of the kidney.

The most important source of *cultures* of amebas in man is the supposedly healthy intestine, and to a less extent the dysenteric one. A number of observers have reported cultivating amebas from both these sources; but of these only a few state that they have grown the true dysenteric ameba, and their work has been severely criticised. Kartulis was one of the first to maintain that he had been successful in obtaining such cultures, and that he had reproduced the disease in cats by inoculation.

Our work in relation to this subject has progressed sufficiently to enable us to state that neither the physical condition of the patient nor the pathologic changes in the colon are the determining factors in the cultivation of parasites from the stool. Several of the amebas which we shall discuss in detail below were isolated from the dysenteric stools in cases of intestinal amebiasis (amebic dysentery), and another was grown from an amebic ulcer, the cultures being made at a fresh necropsy.

Cultural characteristics (general).—Cultures of amebas in general take on an appearance dependent upon the growth of symbiotic organisms, and so far as we have been able to see, there are

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no appreciable differences between cultures from different sources when they are grown with the same organism.

So far as the parasites themselves are concerned, they do not seem to produce any microscopic evidence of their presence in the media, due probably to their transparency, and to the fact that they no not colonize or pile upon one another.

Another noticeable fact is that amebas do not develop below the surface in solid media. For this reason cultures are made in plates containing hardened media. This failure to develop to any extent in the depth is probably chiefly due to the density of the media and not to the partly anaerobic conditions which are present; for when the amebas happen to be below the surface of the medium in association with a liquefying organism, they may multiply, but do not extend beyond the liquefied area.

On the surface of plate cultures, where they may best be studied, the growth and spreading of most of the amebas, under favorable conditions, is quite rapid. By observing such a culture under the microscope, some idea is gained of the relation of the amebas to the bacteria, and the fact, of more or less importance, as to which precedes in culture, may be determined. In many instances the former travel quite rapidly and soon distance any bacteria excepting those directly in their track. Casual observations of plates may reveal them at some distance from other microörganisms. If, however, a very careful search is made, a few bacteria will be found to be following them very closely. Although the amebas may for a time take the lead, the process is not continued, for their movements soon begin to slacken until numerous bacteria have overtaken and even preceded them, whereupon their rapid progress once more begins. Division by fission may be frequently observed during this time. There is one very noticeable condition at this stage, namely, the general course of the amebas is away from the center of inoculation where the masses of bacteria are located, or to express it more exactly, they do not progress toward the bacterial masses. Amebas on the margin of the growth are practically always active, while the encysted forms are found nearer the center.

If one were to judge from the direct course which the amebas pursue away from the bacteria (fig. 3), it would appear as if they were trying to avoid the latter. Yet, as we have already observed, as soon as they are a short distance in advance, there is a slowing of motion until the bacteria again are in the lead, when the amebas

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resume their former activity. This hide-and-seek phenomenon may be repeated by an ameba for hours, although eventually it begins to lag behind, and finally becomes round or encysted.

Morphological characteristics.—The measurements of amebas, as they are given by different authors, vary greatly, as the following brief list proves:

Marshall gives diameters as being from 10 to 40 μ, but usually 24 to 30 μ; Futcher, usually 12 to 26, the average being 20 μ; Quincke and Rose 15 to 25 μ; Kartulis, 12 to 30 μ; Lafleur, 6 to 35 μ, usually 12 to 26 μ, varying much in different cases, but fairly uniform in the same stool; Osler, 10 to 20 μ; Kruse and Pasquale, 10 to 50 μ; Craig, 5 to 35 μ; Zorn, 14 to 22 μ; and Strong and Musgrave, 10 to 50 μ.

After further development of our work with cultures, and a determination of the life cycle, the morphology may become an important point in differentiating species, but at the present time no value can be attached to it. *It is certain that, with our present knowledge, the measurements of amebas from stools can not be used for purposes of differentiation either as to the species or as to pathogenicity.*[1]

It is always easy to transfer amebas from a fluid to the surface of a solid medium, but the reverse of this process is not easily accomplished. Amebas isolated from water or some other fluid substance, after developing for a time on solid media, do not grow well, if at all, when returned directly to the fluid in which they formerly thrived. Amebas obtained from human sources are the most difficult to grow in liquids, and when they are first isolated from the intestine they do not do so at all.

In fluid media the greater proportion of the development at first takes place at or near the surface, but eventually it extends until amebas are found in small numbers throughout the substance.

[1] Morphologically, in cultures, amebas are also variable, the differences depending upon several conditions, such as the age of the parasites, the phase of the life cycle, the density of the media, and other factors of environment. In order to insure the greatest accuracy and to render them of any significance, the measurements should be made only when the animal is in the round stage, and preferably when encysted. Even when these precautions are taken, differences occurring in cultures from the dysenteric intestine will be found to lie between four and forty microns. Apparently there is considerable variation in the cysts grown from a single organism. The measurements of some of our amebas are given under the discussion of special amebas.

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In a natural fluid environment, such as water, they are rarely found in the round or encysted stage, and they are but little more so in fluid cultures artificially prepared. When the conditions are suitable to life, encystment is apparently less frequent than when the amebas are placed in those which are unsatisfactory. Encystment may be brought about in a number of ways, for example, by a change in the reaction of the medium from neutral or slightly alkaline to slightly acid, by an increase in the amount of nutrition in the medium, causing the bacteria to multiply more profusely, by cold, heat, chemicals, etc.

Agglutination phenomena.—Agglutination phenomena have been noticed by Zaubitzer, who, with cholera vibrios, prepared cultures of his amebas grown on straw infusion. Serum from a guinea pig immunized against cholera was then added, and agglutination both of the vibrios and the amebas occurred. Neither the former nor the latter were killed by this process and could subsequently be grown by transplants. The author failed to find similar results when cultures with less toxic organisms were used, and he says that this was probably due to the fact that no satisfactory agglutinins could be obtained.

We have repeated Zaubitzer's experiments, and find as he did, that the agglutinins of a symbiotic bacterium have no destructive action on associated amebas. When the agglutination of the bacteria was brought about by a satisfactory serum all of the amebas did not encyst or become round, some of them continuing their ameboid motion without interruption.

Nutrition.—The means of nutrition of amebas are undetermined. Formerly it was supposed that the engulfing of certain substances within their protoplasm served this purpose, for amebas are known at times to take up red blood cells, bacteria, yeast cells, and other granular material. Recently the idea that these substances serve as food has failed of general belief. Apparently no one as yet has proved that the red blood cells are digested by the amebas, although these inclusions have been observed by different workers for varying lengths of time. Normal red cells have often been seen in their protoplasm, as have also bodies which were supposed to represent the remains of digested ones; but the process of digestion has never been followed out. On the other hand, amebas have been seen to discharge these cells, after the latter have remained within the protoplasm for some time, and these cells were

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apparently still in a normal condition. In cultures the protozoa may often be seen to discharge all the granular material and foreign bodies which they contain just before entering the encysted stage.

Reaction of amebas to physical conditions.—The behavior of amebas towards varying physical conditions is important for several reasons, particularly as a knowledge of their influence will facilitate methods of classification and may throw new light on prophylaxis and treatment.

Drying.—Celli and Fiocca found that cultures of amebas would withstand drying for from eleven to fifteen months. Miller concluded that certain encysted amebas would withstand the same treatment for six years, and that others, particularly those which do not form well-marked cysts, were killed when subjected to this process.

Tsujitani, in order to obtain pure cultures from those mixed with the cholera bacillus, took advantage of the resistance of amebas to drying. Gottstein's cultures were killed on drying.

Zaubitzer's hay infusion ameba would resist drying for sixteen days, and would grow after complete drying when they were transplanted to culture media.

Our experiments on this subject are not completed, but it has already been found that there is a difference in the susceptibility of different amebas towards dessication; but we are not sure that this difference is an intrinsic one. Ameba 11524, after two months in plates which had during that time become dry and hard, grew on transplants; but Ameba 25624, and some others, failed in this respect even when transplanted to media inoculated with the original associated bacterium. We have some amebas which grow well on transplantation from cultures six months old, while others are apparently dead at the end of this time.

Temperature.—The influence of temperature on amebas merits careful consideration.

The optimum temperature for cultures, as reported by different authors, varies greatly, but as a rule it may be said to lie between 20° and 28° C. Some, however, have found 37° C. to be the most satisfactory. Gottstein gives the optimum at 20° to 22° C., the minimum at 10° C., and the maximum at 37° C.

All the amebas which we have so far studied, including those obtained from the intestine as well as from outside sources, flourish profusely at room temperature, but do not grow well at incubator temperature on the one hand and but very slowly at that of the ice box on the other. We have been unable to verify the

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usual statement that amebas always lose their motility at or below 75° F. in stools, and certainly such is not the case in cultures.

As to the maximum temperature which amebas are able to resist, there appears to be some difference between the organisms described by the various authors.

Tsujitani says that cysts are killed by ten minutes' exposure to a temperature of 60° C., Celli and Fiocca consider 45° C. for five hours or 50° C. for one hour to be sufficient to kill them in the ameboid stage, whereas 60° C. for one hour does not destroy them in the encysted one.

Our results with reference to the maximum temperature have shown differences between amebas obtained from different sources.

EXPERIMENTS.

Ameba 11524 multiplied much more slowly in the incubator than at room temperature, and some reproduction occurred in the ice box.

Old encysted cultures of ameba water were exposed to 60° C. for one hour. Transplants to sterile media and to media containing the symbiotic bacteria of the original culture only, showed no growth. Controls produced large numbers of amebas in twenty-four hours.

Cultures of the same ameba, fifteen days old and in great part encysted, were exposed to 50° C. for one hour. Transplants developed no amebas, while the controls gave good growth.

Encysted cultures of ameba 11524, two months old, were exposed to 60° C. for one hour. Transplants to sterile media produced no growth; but in tubes smeared with the symbiotic bacteria of the original culture (*Spr. cholerœ*) amebas developed in forty-eight hours. Cultures ten and fifteen days old and to a large extent encysted gave negative results on transplants after being exposed to the conditions above described. Cultures of this ameba one month old were exposed to 50° C. for one hour. Transplants to sterile media, as well as to media containing the symbiotic bacteria only, developed a good growth in forty-eight hours.

Cultures of ameba 11147, two months old, were exposed to 60° C. for one hour. Negative results were obtained when they were transplanted to sterile media as well as to media inoculated with the symbiotic bacteria of the original culture. Control transplants made just before heating developed a good growth in twenty-four hours. Cultures of the same ameba, one month old, were subjected to 50° C. for one hour with negative results on all transplants.

Nothing is said by those who have cultivated amebas with reference to the minimum temperature which the organisms are able to resist. Kruse and Pasquale, however, were able to produce dysentery in cats by using feces, containing amebas, which had been frozen and thawed; and from this they inferred that dysenteric amebas are probably not destroyed at a temperature somewhat

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below 0° C. There are other observations in literature which indicate that at least some varieties of these parasites are very resistant to cold. The geographic distribution of dysentery, to say nothing of that of amebas in general, indicates that under certain conditions they are able to withstand intense cold. Our experiments with cultures have confirmed this, and have also shown that, as in the case of high temperatures, amebas are not all equally resistant under apparently similar conditions.

EXPERIMENTS.

Encysted cultures of Ameba 11524, two months old, were placed in cold storage at —12° C. for twenty-five days, and growth was obtained on transplant. With a fifteen-day-old culture subjected to similar conditions no amebas developed.

Finally, a culture of the same ameba, three and one-half months old, after being subjected to —12° C. for forty-five days, on transplant gave a growth of amebas, which, however, did not multiply very rapidly until the second generation. In one tube no growth was obtained on the second transplant.

The same experiments were repeated with Ameba 11147 and Ameba "water," but no growth was obtained on any of the transplants with either of these parasites.

Light.—The action of light on these protozoa is of interest because of its influence on certain members of the group, and it is particularly so at the present time when light therapy is attracting so much attention, and because the modern tendency is to explain the action of certain chemicals, such as quinine, on malarial parasites, by its fluorescent or other light effects.

Zaubitzer obtained growths of hay infusion amebas on making transplants from cultures which had been exposed to sunlight during eight to nine hours. However, if they were under similar conditions for an entire day no growth resulted.

Observations which show that red light causes amebas to stream and that light from the opposite end of the spectrum causes them to encyst are very old and have been often repeated. Dreyer has recently gone over this subject again with interesting results. He finds that encysted amebas are very much more resistant to light than those which are not in this condition. When the Finsen light was used the resistance was thirty to thirty-three times as great; with the use of light passed through uncolored glass five to six times; and with light passed through blue glass five times greater. He found that encysted amebas were destroyed in about twenty-five minutes by light passed through rock crystal (ultraviolet); in from sixty to seventy minutes by an equal quantity passed through uncolored glass, and in from seventy to eighty minutes by light transmitted through blue glass. According to his observations, the ultraviolet rays

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are thirteen to fourteen times as powerful in their action as is white light, and eighteen to twenty times as much as blue light.

Schaudin proved that X-rays destroy certain amebas and other animal parasites, after they have been exposed to their influence for some time. Tappeiner and Jesionek demonstrated that fluorescent substances and daylight or sunlight destroy bacterial life as well as the properties of enzymes and toxines.

Dreyer found that the addition of a very small amount of erythrosine to media containing bacteria or infusoria render such organisms very much more susceptible to yellow and green rays. He recommended the application of this principle in light therapy by injecting 1 to 1,000 solution of erythrosine into diseased tissues before their treatment by application of Finsen rays.

Mouton has recently obtained some very interesting results, which in this connection are suggestive. He found that the intensifying of fluorescence in substance of the class mentioned, by the use of various rays, increases their action on certain cells, and suggested a therapeutic application of the phenomenon.

Our experiments with the action of light on amebas are not yet far enough advanced to justify very many definite statements.

Direct sunlight undoubtedly has at least an inhibiting influence on them. When fresh transplants of Ameba 11147 were exposed to sunlight for three hours no development occurred; but motile cultures of Ameba 11524, twenty-four hours old, were not killed by three hours' exposure to the sun's rays.

X-rays also exert an unfavorable influence on cultures. It was found that old encysted amebas would grow out on transplant after twenty minutes' exposure to this light. On the other hand, when young, growing cultures were exposed to it for twenty minutes, many rounded and encysted forms were observed and growth was frequently delayed on transplant.

However, the most interesting of the light phenomena is the action of fluorescence upon these protozoa, and we regret our inability to say more than the following about it at this time.

A young, growing slant culture of one of our amebas was placed inside a large tube containing a solution of fluorescein, so that the inner tube, which contained the culture, was entirely surrounded by the solution. This preparation, together with a control culture of the same age, was then placed in the direct sunlight for two hours. On microscopic examination all the amebas in the tube surrounded by fluorescein were found to be encysted. Transplants from the control tube gave a good growth, but those from the experimental one remained free from amebas.

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Similar experiments have been made, using various fluorescent substances, such as quinine bisulphate, orcein, eosin, fluorescein, and resorcin, with different amebas as well as with growths of the same organism of different ages. Some of these substances, particularly quinine bisulphate, fluorescein, and orcein, under the conditions named, certainly, to a considerable extent, exerted an inhibiting influence on the amebas. This was also found to be true when the parasites surrounded by these solutions were exposed to the action of X-rays.

Theoretically at least, we should expect to secure the best results by exposure to ultraviolet rays concentrated through fluorescent substances; but the ultraviolet rays for experimental purposes are at the present time not available in the Philippine Islands.

Reactions to chemical agents.—In view of the therapeutic application of chemical agents, the reaction of amebas towards them is of the highest importance.

Perhaps the substance most generally mentioned in this connection is quinine, this being the one most universally used in the treatment of intestinal amebiasis (amebic dysentery). Lösch first noticed the parasiticidal action of the drug, and his observations have since been confirmed by several experimenters in so far as they refer to the action of quinine on amebas in the stool. The dilution supposed to have the most efficient action is variously given, the proportions ranging from 1 to 300 to 1 to 1,000. Some authors have expressed doubt as to whether quinine possesses any specific action.

EXPERIMENTS.

A slant culture of Ameba 11147, twenty-four hours old, was washed with a 1 to 2,500 solution of quinine hydrochlorate. The amebas quickly encysted, and in from five to eight minutes many of them had broken up and disappeared. Ten minutes afterwards no development of amebas occurred in cultures from this emulsion, although the bacteria showed satisfactory multiplication.

This experiment was repeated with similar results with Ameba 11524. However, a scant growth of amebas was obtained when transplants from the emulsion were made ten minutes afterwards.

Forty-eight-hour-old plates of Amebas 11147 and 11524 were exposed for ten minutes to formalin vapor. Transplants failed to develop amebas.

Encysted cultures of Ameba 11524, two months old, were washed off with a 1 to 1,000 solution of formalin. The parasites were quickly encysted; but in twenty-four hours a small number of amebas developed on transplants which had been made from the emulsion five minutes after exposure. When cultures of Ameba 11147 of the same age and under similar conditions were used, identical results were obtained; but when Ameba "water" was employed, no growth was obtained on any culture.

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Old and young living cultures of Ameba 11524 were washed off with carefully neutralized solutions of acetozone of 1 to 1,000 to 1 to 10,000. In some instances the parasites were encysted; but, even when the 1 to 1,000 solution was used, they grew out on transplants made after five minutes.

With the use of carefully prepared solutions of acetozone, *1 per cent acid to phenolphthalein,* the findings were entirely different. This slightly acid solution produced results which were most satisfactory and out of all proportion to any difference attributable to the small amount of acid present, which of itself would exert but a very inconsiderable inhibiting influence upon the protozoa.[1]

EXPERIMENT.

Ameba 11524, in cultures of all ages, from those of young motile ones to those encysted for six and one-half months, have been washed with solutions of 1 to 5,000 to 1 to 2,000 of this slightly acid acetozone, and in every case have given negative results on transplant (after ten minutes) to sterile media as well as to media smeared with the satisfactory symbiotic bacteria.

A phenomenal resistance to alkalies has been noticed by several observers. All our cultures, whether young or old, motile or encysted, were killed by 20 per cent solutions of sodium hydroxide or potassium hydroxide, as demonstrated by their failure to develop on transplants made after five to fifteen minutes.

EXPERIMENT.

Ameba 11524 was transferred to media with varying amounts of sodium hydroxide and potassium hydroxide in order to determine the maxi-

[1] There may be two explanations of the difference between the action of benzoyl-acetyl peroxide (acetozone) in alkaline or neutral solution and in acid. The first is that, if the solution is alkaline, it is not improbable that the acetoperacid, produced by the hydrolysis of benzoyl-acetyl peroxide, either liberates a minute quantity of chlorine from the chlorions or, as seems more likely, is able to oxidize the chlorious to hypochlorite, the latter having an energetic oxidizing action. The second would be that acetoperacid, or benzoperacid are much more rapidly decomposed, with the formation of hydroperoxide and the salts of acetic and benzoic acids, in alkaline than they are in acid solution. However, hydroperoxide has very feeble germicidal properties and hence, probably also a feeble action on protozoa, whereas the per-acids are intensely germicidal. Therefore in the great dilutions which are used, the alkali might very readily so hydrolyze the per-acid that it would practically be inert, whereas its full effect would still be present in the acid solution.—FREER.

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mum degree of alkalinity in which they would propagate. 1 to 5 cubic centimeters of N/10 solution of these chemicals were added to tubes containing 10 cubic centimeters of media neutral to phenolphthalein. We were unable to secure a good growth on cultures which contained more than 3 cubic centimeters of the alkali.

However, what is of more importance, from a practical standpoint, is the proportion of acid which may be present and still allow these parasites to propagate. They are very susceptible to the action of acids, but, as we have determined, some of them multiply in media which are more acid than is usual in the normal stomach.

Ameba 11524 grew in a medium $\frac{1}{30}$ per cent acid (HCl) to phenolphthalein. By starting with a neutral medium and a symbiotic organism, like *B. coli,* which multiplies well in acid media, and by gradually increasing the acidity on successive transplants, multiplication may be obtained on a medium even more strongly acid than this.

Sera, blood toxins, etc.—An understanding of the action of these substances is essential to a proper knowledge of the infection produced by amebas, and we have therefore begun some preliminary work on this topic.

Emulsions from Ameba 11524 in a pure culture of *Spr. Choleræ* were treated in the hanging drop with equal parts of a serum with a high agglutinating power for the latter organism. The bacteria were very promptly agglutinated, and some of the amebas assumed a round form after a few minutes. However, many of them remained motile for one hour and grew on transplant at the end of this time.

A dog was given frequent subcutaneous and intraperitoneal injections of a culture of Ameba 11147 and *B. coli.* At the end of two weeks this animal's serum had no parasiticidal action on amebas, which developed when transplanted to a plate the surface of which was smeared with its serum.

A monkey was given in the abdominal cavity, on every fourth day, injections of Ameba 11147, together with a yellow pigment-producing bacillus. Five days after the last injection the serum of the monkey was found to have no unfavorable action on amebas in the hanging drop, and transplants developed on plates smeared with it. There was no variation from these results when the experiment was repeated with Ameba 11524.

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When a few drops of human blood or a small amount of human serum were added to fluid cultures of Ameba "water" of the first few generations, the parasites all encysted in a very few minutes and refused to grow when transferred to plates on which the surface of the medium had been smeared with human serum. When similar cultures of these amebas were injected subcutaneously or into the liver of a rabbit or monkey, the protozoa which were reclaimed by culture from the abscesses were not so susceptible to the action of human blood or serum as they had been. When cultures of amebas from straw infusions were used, the results were similar to the foregoing; but with those of Ameba 11524 no apparent effect was produced by the introduction of human serum and good growth was obtained on transplant. This demonstrates the influence of animal tissues upon the adaptability of these parasites, a property which can still further be verified by cultures.

If to cultures of Ameba "water" very minute quantities of serum or blood held in solution by potassium citrate are added, and the amounts are gradually increased on successive transplants, a culture may eventually be obtained, which flourishes nothwithstanding the presence of a considerable quantity of blood or serum. The addition of the blood, however, enriches the media to such an extent that the bacteria develop very rapidly, and to avoid this it is advisable to make alternate transplants to those free from blood.

Cultivation of special amebas.—We purpose here to discuss briefly, under their laboratory numbers, a few of the special amebas which we have cultivated. In order to show conclusively that we are dealing with amebas which cause dysentery, a brief description of the clinical manifestations of the cases is also introduced.

Ameba 11524 was isolated from a dysenteric stool. The patient, an American nurse, had been suffering with intestinal amebiasis (amebic dysentery) for about one year and amebas had repeatedly been found in her stools during that time. Her treatment was of an intermittent character, being suspended with the subsidence of clinical symptoms. The course of the disease was the usual one, with very chronic tendencies and with frequent and sometimes quite severe exacerbations.

Our first cultures were made during such an exacerbation, and at a time when there could be no reasonable doubt as to the correctness of the diagnosis. From a specimen containing many motile amebas, from 20 to 35 μ in diameter, and carefully taken in a

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manner which prevented outside contamination, a large number of cultures were made as follows. Fifteen sterile Petri dishes were charged with our special medium, which was then allowed to harden. The surfaces of twelve of these plates were then smeared with pure cultures of different bacteria from stock cultures, including those cultivated from the intestine in other cases of amebiasis. All the plates (including the three sterile ones) were inoculated with the stool containing the amebas by merely smearing small quantities over the surface of the plates with a platinum loop.

These cultures were examined at the end of twenty-four hours and no amebas were found; but on a second examination, forty-eight hours after inoculation, two out of the fifteen contained large numbers of motile protozoa. These two cultures were identical, being symbiotic with a bacillus (9650b) which has already been described. Examinations made as late as seventy-two hours after inoculation revealed no amebas in the other cultures.

Transplants with the first, second, and third generations, as in the first case, were successful only with media which had been smeared with Bacillus 9650b. Transplants from the original culture made on the fifth day failed to grow even under these conditions; those from the second generation after the same interval showed a growth, but none occurred on those made on the tenth day. The fourth and subsequent generations developed by transplanting to sterile media, and only those bacteria which were carried over in the loop during the transfer were present.

Experiments with this ameba on other media have been unsuccessful. At best but a scanty growth has been obtained upon somatose agar, and reproduction on transfer to various fluid media has been scarcely more satisfactory. We have, however, by gradual reductions in the density of the substrata, succeeded in obtaining good growth in fluid media composed of beef extract 0.5, salt 0.5, water 1,000, made with a reaction 1 per cent alkaline to phenolphthalein.

Transplants to old cultures of stock bacteria grown on ordinary laboratory agar have not given satisfactory results. On such cultures encysted amebas could be found among the bacteria for several days, but there was little or no evidence of multiplication. Five months after this ameba had been isolated and had become well accustomed to artificial media, it gave growth when transplanted from a culture with comparatively few bacteria to a

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sterile ordinary laboratory agar slant; but as the bacteria on this medium increased, the amebas which were transferred were soon lost.

Growth was found to be very satisfactory for a long time on a medium composed of 2 per cent agar and 0.5 per cent of beef extract (1 per cent alkaline), the development decreasing only when a marked diminution in the number of bacteria, which is usual with this medium, occurred.

Microscopically this protozoon, as obtained from culture, is indistinguishable from those seen in the stools of the patient, and it is a true dysenteric ameba. Its measurements in the round stage in the stool were 25 μ to 35 μ, and those in the cultures generally corresponded with these figures, but they varied greatly, owing, no doubt, to environment and the phase of the life cycle at the time of the examination.

Encystment of the ameba, when it was in unsatisfactory environment, was very rapid, and when once this stage was reached, no further reproduction could be observed until it was transplanted. On suitable media multiplication by fission was frequently observed, and motile amebas often remained present in the cultures for days. However, the final result always was encystment, which began at the point of inoculation and progressed toward the outer margins of the spreading growth, where the youngest parasites were the last to undergo the change.

A very interesting feature of these amebas is the variation in the character of the pseudopodia, which ranges from a decided lobose to the most exquisite spinose. These differences, at least in part, depend upon physical environment.

In our collection there now are cultures of this ameba which were started from a single parasite. They are in pure culture with four different bacteria—*B. coli, Spr. choleræ* and two different pigment-producing saprophytes. The protozoa grows well with all these organisms, and by methods already given has been changed from one to the other and *vice versa.*

In one instance, dysentery in man followed the ingestion of three gelatin capsules filled with scrapings from the surface of cultures of this ameba in symbiosis with a harmless bacterium. Dysentery has also been produced in monkeys by similar cultures as well as with others where the bacterium in symbiosis was a pathogenic one.

Ameba 11147 was isolated from the stool of a male Filipino,

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who had suffered from recurrent attacks of intestinal amebiasis for about five months, and who had apparently recovered under treatment. The diagnosis of intestinal amebiasis was confirmed by post-mortem examination three months later.

The technique used in isolating this ameba was identical with that described for Ameba 11524, and the only growth obtained was on plates which had been smeared with the same bacillus as in the first case.

The second generation of this organism grew on sterile plates, to which only those bacteria which were carried over in making the transplants were added; and no further trouble has since then been experienced in keeping satisfactory cultures. On three different occasions cultures were obtained from single ameba. No differences were apparent in these three cultures, and we therefore concluded that the original one was of a single species.

Cultures from a single ameba were then transferred to pure ones of several bacteria, including *B. coli, Spr. choleræ,* and two pigment-producing saprophytes. The growth with all these organisms was good, after it had once been obtained. The symbiosis of this ameba has been changed from *Spr. choleræ* to a yellow-pigment-producing saprophyte, obtained by exposing plates to the air and back to *Spr. choleræ* on two different occasions.

Attempts to cultivate this organism on other media gave results very similar to those already described for Ameba 11524. As with the former and all others, an examination of cultures will serve to distinguish the unsatisfactory media, in which the amebas encyst in a very few hours, with no further development until they are transplanted to a more favorable environment; whereas, in suitable media multiplication continues for days before encystment (the final result in all cultures). When the latter stage is once reached, no further development may be expected until transplants are made. These are successful in the interval from one week to at least seven months.

The longevity of the amebas, not considering the probable differences in species and the length of time during which they have been kept on artificial media, is apparently influenced to a certain degree by the class of microörganisms present in symbiosis; for while all transplants from old cultures with one bacterium may give good results, those from similar cultures with another bacterium may be negative.

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Ameba 11147 is apparently pathogenic. In the stools is measures from 22 μ to 28 μ, its average in culture being about the same. As has been found true of all our cultures, however, there is considerable variation in size, depending partly no doubt upon environment and partly upon phases of development. The pseudopodia thrown out by this organism in culture are of such a character as to place it in the lobose variety; but under certain conditions, not yet fully understood, but in all probability depending upon the density, viscosity, and other physical properties of the media, they may be of another type, including the spinose. In cultures this ameba has been repeatedly observed to multiply by fission, and it apparently has distinctive characteristics. Dysentery has been produced in monkeys by feeding old cultures of it in symbiosis with both pathogenic and nonpathogenic bacteria.

Ameba "water" was isolated from the water supply of Manila, from which, during a considerable portion of the year, these parasites may be obtained almost at will. We have made numerous cultures of amebas taken from taps in the city and from the Mariquina River, the source of the water supply, at various places along its course from the origin to the intake. However, in this report we shall consider only one individual, which was isolated about a year ago and has been cultivated on artificial media since that time.

The culture was started in two days. First, some water was centrifuged and the deposit from the bottom of the tubes was transferred to sterile plates prepared as already described; amebas developed at the end of twenty-four hours. By the other method, which is the one which we now use in our routine work for the cultivation of amebas from water, or other outside sources, 200 cubic centimeters of water was drawn into a sterile flask, to which was added 0.5 to 1 cubic centimeter of ordinary laboratory bouillon (1 per cent alkaline to phenolphthaleïn) for each 100 cubic centimeters of the sample. After from twenty-four to seventy-two hours amebas may be found on the surface of the fluid, and with a platinum loop transferred to plates.

This ameba, as is true of all our water amebas, grew well from the beginning without the addition of bacteria other than those associated with it. At the time of the transfer of this organism from the fluid to the solid medium, it was associated with several other varieties of parasites, mostly *ciliata,* some of which endured through one or two transplants on solid media, but at length

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entirely disappeared. For some time after we began its cultivation, it grew readily on a variety of fluid media, including most of those recommended by previous experimenters; but after some months on solid media it became much more selective, and it is now quite difficult to transplant it directly to any fluid. Shortly after its isolation a pure culture—so far as the amebas are concerned—was secured by the methods already discussed. Later it was grown with pure cultures of several organisms—the "pure mixed culture" of Frosch.

In its behavior on other media this parasite resembles the two already described. Of all our amebas which form true cysts, this one is the most susceptible to the action of a luxuriant growth of bacteria. When placed in suitable medium and under proper conditions of symbiosis, it lives for a long time; but with an unsatisfactory bacterium, transplants may not grow for two weeks. We have succeeded in transplanting it from favorable tubes after five months, and again we have entirely failed to note a growth from cultures one month old.

Morphologically this ameba closely resembles Ameba 11524, but it apparently has certain differences. Its size varies considerably, evidently under the influence of certain physical conditions as well as of others which we do not as yet fully understand. Full grown amebas at rest or encysted measure from 15 μ to 35 μ.

This ameba we believe to be capable of causing dysentery, for we have produced a disease which is clinically and anatomically identical with it by feeding old cultures to monkeys.

Ameba "lettuce" was isolated from a head of lettuce grown on the agricultural experimental farm in Manila. The technique used in its cultivation closely resembled the one employed for water amebas. The lettuce was shaken with water in a sterile flask. Afterwards the water was decanted into other sterile ones, and the washings repeated four times. Amebas were present in all of the cultures from the decanted fluid. As was true of water amebas, the organisms from lettuce grew from the beginning without the addition of special bacteria, and cultures have been continued on solid media during a period of five months. From a single ameba, cultures have been obtained with only one variety of bacteria. The lettuce ameba is somewhat more restricted in its selection of symbiotic organisms than are some of the others. In size it shows great variation and its cyst formation is of an indistinct type. Favorable

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cultures of this ameba were fed to three monkeys, and amebic dysentery was produced in two cases.

Ameba 25624 was isolated from the stool of a Filipino, who gave a history of intermittent diarrhea with occasional exacerbations covering an interval of three months. Mucus and blood were present in the stool during such times. The patient's evacuations at the time of the first examination were semiliquid and contained a considerable amount of mucus with a fair number of red blood cells. Two careful examinations, however, failed to demonstrate the presence of amebas.

Cultures from one of the specimens, the precautions being the same as with Ameba 11524, gave positive results on two plates—one of sterile media inoculated directly from the stool and the other previously smeared with a pure culture of the yellow pigment-producing saprophyte described under Bacillus 12935. The plates which had been inoculated with Bacillus 9650b, which it will be remembered was the satisfactory organism for both Ameba 11524 and Ameba 11147, gave negative results. No difficulty has been encountered in keeping this ameba alive on transplants, and it is now one of our stock cultures.

This is one of the most interesting parasites which we have cultivated, and in a general way it closely resembles ameba lettuce. More care is required in its propagation, as it is particularly selective as regards symbiotic bacteria. In comparison with some of the others, it will not remain alive as long without transplanting, and in many ways it shows itself to be a more delicate organism. It has been seen to multiply by fission on plates, but it is certain that another method of division exists. Judging from this as well as from its condition when at rest, we feel sure that it is a distinct variety and also that it is an ameba capable of producing dysentery.

Ameba "C" was isolated from the stool of a native, who at the time we secured the culture denied having had dysentery or diarrhea. This patient, together with a number of others, all of them with negative histories of diarrhea or dysentery, passed stools which on examination contained fair numbers of amebas after a cathartic dose of Carlsbad salts. At the time of examination the patient in question was in the hospital because of a fracture of the leg, and his bowels remained normal for eighteen days, when diarrhea developed and with the usual intermissions soon reached

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a stage where a diagnosis of intestinal amebiasis was justifiable on purely clinical grounds.

The methods used for the culture of this ameba were the same as those already described for the other intestinal amebas. They did not develop on any of the sterile plates inoculated directly from the stool, and only two of those previously smeared with bacteria gave a growth. In all its cultural manifestations this ameba closely resembles Ameba 11147, which has already been described.

Ameba 34354 was obtained from the stool of a patient suffering from dysentery. In this case the patient died and the same ameba was grown post-mortem from ulcers of the colon. The case was that of a private patient of Dr. Strong, to whom we are indebted for the clinical and post-mortem data used. The patient was an American, whose dysentery, at the time of his death, was of about seven months' duration, treatment having been uninterrupted during that time.

Some three months before death we completely failed to grow amebas from a specimen of his stool, though microscopically it contained large numbers of these parasites embedded in bloody mucus; and on the day before death another attempt was made. A large number of plates were inoculated, amebas being obtained from three; one of these was a sterile one, inoculated directly from the stool, and the other two had been previously smeared with bacteria which had formed satisfactory symbiosis with other amebas.

At the necropsy we made a large number of cultures from the ulcers of the colon caused by amebas and from two of the several liver abscesses. For the intestinal ulcers amebas were successfully grown, but not from the liver abscesses.

No discussion of this organism will be given at the present time, as it has been too recently isolated.

Ameba 17786 was isolated from the stool of a patient who had probably suffered from amebic dysentery during several months, but who had developed clinical symptoms for thirteen days only. Plates, with and without bacteria, were made in large numbers, the organism developing only on one which was sterile and inoculated directly from the stool.

Ameba 12485 was cultivated from the stool of a member of the Laboratory staff who had been in the Philippine Islands five months, but the diarrhea was of only two days' duration at the time the cultures were made.

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The plates were made both with and without bacteria in the usual way, and a satisfactory development of amebas was secured in several, including both those free from special bacteria and those to which such organisms had been added.

Notwithstanding treatment was promptly instituted, the patient continued to have exacerbations, and at the present time, six months after the first cultures were made, still has decided symptoms of dysentery.

On two subsequent occasions we have failed to cultivate, on sterile media, amebas from specimens of this patient's stool. Experiences similar to those in this case have also been encountered in others often enough to justify the conclusion that *as a rule* the earlier the stage of the infection, the oftener will ameba grow when specimens of feces containing them are inoculated directly to sterile media.

Ameba 12483 was grown from the stool of an American who had been suffering with intestinal amebiasis for about six months. At the first attempt no amebas were obtained, although a large number of plates were used and inoculations were made directly from the stools upon sterile media and upon that previously smeared with a number of bacteria which had proved satisfactorily symbiotic with other amebas. After this failure, agar-plate cultures were made from the stool, all the colonies which grew were isolated in pure cultures, and the amebas were successfully cultivated on media smeared with some of these bacteria. No growth occurred on any of the controls, which were prepared as in the first instance. On working out the bacteria, whose symbiosis furnished the positive results, it was found that they were all organisms of the colon group, and apparently identical. These amebas were quite selective for this bacillus for several generations, but later could be satisfactorily grown with a number of organisms. In its general cultural characteristics it closely resembles Ameba 11147.

Ameba "A" was grown from the dysenteric ulcers of a patient who had not shown clinical evidence of the disease during life, and who died of an intercurrent lobar pneumonia. This patient, a native, was an inmate of Bilibid Prison, and upon admission to the hospital amebas were found in his stools, although he had no clinical symptoms of dysentery and failed to give a history of previous attacks. Up to the time of his death, nine days after admission, no bowel trouble had developed. At necropsy, which was performed three

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hours after death, the classical lesions were found in the coecum and the ascending colon, and to a less extent about the splenic flexure. Made with the usual technique, cultures from the floors of the ulcers gave amebas on two plates which had been smeared with *B. coli.*

Ameba "L" was cultivated from the stool of a patient on the second day of the disease, as determined by clinical symptoms and history. It grew well from the first, both on sterile media which had been inoculated directly from the stool as well as on that in which *B. coli* had been used as a symbiotic organism.

Ameba "G" was grown from the stool of a young lady who at the time was not suffering from diarrhea and gave no history of such trouble. The culture was made on the day following a rupture into the bowel of an ischio-rectal abscess. From that time the patient developed clinical symptoms of amebiasis with the presence of amebas in the intestinal contents.

The amebas grown from this case are slightly below the average size, although in the round stage on favorable cultures some of them measure 25 μ. They are not as viable, and, as do most of our cultures, require frequent transplantation.

Ameba "urine" was grown from urine drawn aseptically from the bladder of a man suffering from hemorrhagic cystitis. This case was under the care of Dr. J. R. McDill, and will be reported fully by him at a later date.

Further work may, and probably will, show that some of the amebas which we have described are identical, and for this reason we have avoided naming or individualizing them further than by attaching their laboratory numbers for convenience in discussion.

On the other hand, judging from what has already been learned of them, there would seem to be more than one variety. When we remember that two of the most important ones which show particular differences are from the amebic colon and that the third is from the city water supply, the multiplicity of dysentery-producing amebas is, to say the least, very strongly indicated. If this is fully determined to be the case, our nomenclature will need some revision, and the first ameba shown to comply with Koch's laws should retain the old name *Ameba coli.*

III. ETIOLOGIC SIGNIFICANCE.

The etiologic importance of the amebas in the intestinal and other infections with which they have been found associated has

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been a most debated question almost since the time of the appearance of Lösch's report in 1875. For some time after the publication of his article the literature was meager, but those who reported finding of amebas in dysenteric stools considered them, as Lösch believed them to be, the cause of the disease. The work of Cunningham, Grassi, and others, reporting the discovery of amebas in the stools of healthy persons and of those suffering from other diseases, led to the conclusion by some writers, that these protozoa probably have nothing to do with the production of dysentery. The discussion has never been fully settled. The literature on the subject is too voluminous to review, but it may be briefly discussed under the four headings given below, before taking up the results of our own work, which shows that amebas are the etiologic factor in this form of dysentery.

(1) Amebas are harmless commensals.
(2) They intensify or alter the lesions already present.
(3) There are pathogenic and nonpathogenic amebas.
(4) All amebas are, or may become, pathogenic.

The first of these propositions has had many supporters, from Cunningham in 1881 to Duncan in 1902, the arguments advanced having varied but little. The most important, and the one almost always brought forward, is that amebas have been found in healthy persons. The evidence of the truth of this assertion is based upon a large number of observations, chiefly those of Grassi (1879); Lewis and Cunningham (1881, during a cholera epidemic in India); Calandrucio, Kruse and Pasquale in Italy, the latter authors finding amebas in their own normal stools and in those of twenty healthy persons out of a total number of thirty-five examined; Schuberg (1893), who, after the administration of Carlsbad salts, found amebas in the stools of about ten out of twenty healthy persons; Celli and Fiocca (1895), who isolated amebas from the intestinal canal of two out of fourteen healthy infants, and from three boys in Alexandria after the use of Carlsbad salts (they also cultivated *Ameba coli* from the stool of a healthy infant); Fiori (1896), who encountered encysted amebas in normal stools and motile ones after the administration of salts; Ciechonowski and Nowak (1898): Strong and Musgrave (1900), who found amebas in 4 per cent of a series of specimens obtained after the administration of Rochelle salts to soldiers not suffering from dysentery or diarrhea; Huber (1903), who encountered them in about twenty per cent of the cases examined; while, on the other hand, Janowski, Dock, Zorn, and many others have failed to find amebas in the healthy intestine.

When critically reviewing literature, but little of it seems to be conclusive. All the positive observations except part of those of Celli and Fioca and one of Strong and Musgrave have been

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determined from single examinations, without being confirmed by the subsequent histories of the patients, and from what we know now of the nature of the disease, they are of but little value. Celli and Fiocca, in reporting the presence of amebas in three boys in Alexandria, say that there was no history of subsequent bowel trouble, but they do not give the period during which these observations were continued. One of Strong and Musgrave's patients remained well for about three months, at the end of which time he passed from observation. This period, as we shall show below, was not of sufficient length to prove either that the bowel was healthy or that the amebas were harmless in this case.

The statement of Kruse and Pasquale that amebas were found in their own normal stools probably proves nothing more than that these parasites may be transiently present in the normal bowel, unless they made repeated observations over a longer period of time or did not subsequently suffer from intestinal trouble. On this subject their article makes no statement.

It is thus seen that there are but few authentic accounts of even the transient appearence of amebas in the stools of supposedly healthy persons. Post-mortem examinations have given no different result, because, so far as we have been able to determine, no records of amebas being found post-mortem in a normal intestine in cases in which during life amebas have persisted for any length of time, or indeed have propagated in the intestine.

Finding amebas at necropsy in the intestine without microscopic lesions is not conclusive evidence that such amebas were harmless, even though the parasites were shown to be present in the stools during life for a time less than the maximum period of incubation, which may be more than five months. Such observations, if made without a study of microscopic sections, are still further open to criticism, for there is a preulceration stage in the infection. Owing to an epidemic of pneumonia among the inmates of Bilibid Prison, where amebiasis is also quite prevalent, we have been able to study these early lesions quite extensively, and the findings have fully justified our criticisms of reports which pronounce an intestine containing amebas healthy without a microscopic examination of sections. *We do not wish to state that amebas are never present in the normal colon, for such may occasionally be the case;* but, after considering what has been said above, one is justified in saying that even their transient presence

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in the normal human bowel is a much less frequent occurrence than it is generally stated to be by authors, and that the evidence brought forward in support of the conclusion is not free from criticism.

Whatever may be said of the transient presence of amebas in the normal intestine, their harmless persistence and proliferation there for a time equal to the greatest known incubation period of the disease has not been demonstrated. The observations of Celli and Fiocca in the case of three boys, and the one of Strong and Musgrave, which has already been mentioned, do not fulfill the requirements, and are therefore not conclusive.

To illustrate the incubation period in this infection and in general to see how far the arguments submitted above may be substantiated, the following is offered:

Large motile amebas were found in the stool of a 3-year-old boy, who gave no history of past or present diarrhea and who was apparently in good health at the time of examination. Amebas continued in the stools and the boy remained in good health until the one hundred and eighteenth day after the first examination, when a slight diarrhea developed, and with the usual intermissions progressed to the true clinical dysentery; and he was placed under treatment on the one hundred and twenty-fifth day after the amebas were first found.

A native boy, 18 years old, came under observation for fracture. His stools contained fair numbers of amebas, but there was no history of diarrhea and his bowels remained normal for twenty-two days after admission to the hospital. On the twenty-third day slight diarrhea developed and continued for two days. Shortly after this he contracted plague and died, and at the post-mortem examination the colon showed typical lesions of amebiasis which must have existed for months.

We repeatedly found amebas in the stools of a man in very good health during a period of nearly five months. During this time there had been a few slight attacks of "indigestion," some loss in weight, and occasionally slight indefinite abdominal pains, but no diarrhea. During the sixth month diarrhea developed and gradually became more severe, until the diagnosis of amebiasis was fully borne out by the symptoms. He is still under treatment.

Amebas were found in the stools of five out of forty apparently healthy natives after the administration of Carlsbad salts. One of the positive cases was lost sight of; three of the others were undoubtedly suffering with amebic dysentery at the time of the examination, a fact which became more evident as the disease progressed, because blood and mucus finally appeared in their stools, so that they untimately were placed under treatment. The remaining individual continued to have normal passages and developed no symptoms until the eighteenth day after the first examination, when he suffered with diarrhea for two days, the dejecta containing

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numerous amebas but no blood. He had no further trouble until the twenty-sixth day, when there was a second light attack of diarrhea lasting about the same length of time, amebas as well as a few blood cells still being present. After this the attacks of diarrhea became more frequent and severe, the case gradually merging into a condition which left no doubt of the diagnosis of intestinal amebiasis.

The stools of 300 miscellaneous prisoners in Bilibid Prison were examined after the administration of Rochelle salts and amebas were found in 101 of them. Sixty-one of the positive ones were suffering with dysentery, but the other 40 gave no history of past or present diarrhea.[1]

During the next two months 8 of the latter died of intercurrent diseases, and satisfactory evidence of amebic infection was present in all. A second examination of the remaining 32 made two months after the first showed 26 positive and 6 negative for amebas, and 15 of these were suffering from dysentery.

Thus during two months of observation of 40 patients who had amebas in their stools without symptoms of dysentery, 8 died with lesions of the infection, 15 others developed dysentery, and the remaining 17 are still under observation.[2]

Finally, if we admit, a point unproved as yet, that amebas may be transiently present in the normal colon or even that they may propagate there, it is not proof that amebas are harmless. Such a conclusion would be equally applicable to others of the infectious diseases with generally recognized etiology; it leaves natural immunity and other factors out of consideration, and most important of all takes no notice of the influences which environment may have on the parasites themselves.

Clinical observation furnishes good evidence that all these factors play a part in amebic as in other infections. For example, the disease in children and natives is much less severe and more amenable to treatment than in adult foreigners, and in the former we have seen more than one instance of recovery without treatment. The influence of the environment of the amebas with reference to their pathogenic action has already been mentioned in another part of this paper.

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[1] The average native's statements are useless for purposes of clinical diagnosis, and particularly so with reference to intestinal troubles, from one to five stools in twenty-four hours being considered normal by many of them.

[2] In a third examination made six weeks after the above, 2 of the 17 patients had been discharged from the prison and the remaining 15 were positive for amebas, and clinically the diagnosis of dysentery was perfectly apparent in all of them.

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The next most important argument generally submitted that amebas are harmless commensals is that they have been reported in the intestinal contents of persons suffering with diseases other than dysentery.

Such reports have been rendered by Cunningham, who found amebas in 18 per cent of the stools of cholera patients in India; Celli and Fiocca, in the intestinal contents of twenty-four out of sixty-four infants afflicted with intestinal diseases; Ciechanowski and Nowak in tubercular ulcers of the intestine; Berndt (1894), in subphrenic abscesses following typhoid fever; Gasser (1895), in stools of five out of eight patients suffering with chronic diarrhea following dysentery; Normand, in colitis; Bizzozero, in chronic prostitis; Massiutin, in typhoid, pallegra, acute and chronic intestinal catarrh, chronic diarrhea, colitis following tumors of the colon; Grassi, in diarrheic and dysenteric intestinal diseases; Perroncito, in chronic enteritis, complicated by diarrhea; Babes and Zigura (1895), in fifteen cases of entero-hepatitis; Borchardt (1896), in stools of a patient who had been suffering with intermittent diarrhea for six years; Casagrandi and Barbagallo, in several other diseases than dysentery; Kruse and Pasquale; Quincke and Rose; Schuberg and others.

In looking over this literature one is struck with the fact that nearly all of the diseases mentioned above involve the colon—indeed, the majority as diagnosed are principally of this organ, and further with the fact that most of the observations were made in places where amebic dysentery was undoubtedly endemic at the time.

We have given particular attention to this subject for a number of years in a country where amebiasis is endemic; and while we have repeatedly found amebiasis associated with a variety of other diseases, we have not found in such observations evidence of the harmlessness of amebas. For example, during the late epidemic of cholera in this country amebas have been found in the dejecta of cholera patients, and in some of these cases which were examined post-mortem, the lesions of an old amebic dysentery were found in addition to those of cholera.

The same may be said of typhoid fever. We have complete records of a number of cases of typhoid fever and amebic dysentery in the same patient, in some of which amebas were found in the stools during life, and the diagnosis of a double infection confirmed at necropsy.

With reference to Grassi's often-quoted statement that he found amebas in the stools of patients suffering with colitis, secondary to intestinal tumors, we may say that we have had a case of carci-

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noma of the cecum in which amebas were found in the stools, and a diagnosis of double infection was established at necropsy.

We are justified, therefore, in concluding with Lafleur and others that many if not all of these cases were based on a faulty diagnosis rather than that the amebas were present in these diseases as harmless commensals. The absence of post-mortem observations is particularly noticeable. To draw the conclusion that the amebas present in the diseases reported were not playing a pathogenic rôle without observation after death must be accepted by those fully acquainted with the nature of the disease as almost entirely valueless. Even were such observations made, the conclusions would be but slightly more justifiable, unless it were shown that the amebas had been propagating in the bowel during life, for, as has already been said in discussing amebas in healthy persons, it is only the *persistence* and not the accidental passage of these parasites through the colon that can have any weight in the consideration of this subject.

Amebas secondary in action.—The opinion that amebas are not primarily pathogenic but may possess the power to alter or intensify the lesions already present in the intestine, is one which was formulated many years ago and which has had not a few ardent supporters; and many able men are still of this opinion.

This idea lends itself readily to the conclusions which may be drawn from many anatomic pictures which, without attributing to amebas some pathogenic action, can not be explained by those who have attempted to prove the innocence of the parasites.

One of the arguments most commonly employed to support this position is the statement that amebas may be found in the ulcers and lesions caused by other well-known etiologic agents. A number of writers call particular attention to their presence in tuberculous ulcers of the intestine, and we can confirm this observation at least to the extent that tubercle bacilli and amebas may be present at the same time in intestinal ulcers.

We have recently performed a necropsy on such a case. There was advanced tuberculosis of the mesenteric glands and of the intestine. The intestinal ulcers in their gross appearance more closely resembled those of intestinal amebiasis, but both microscopic and histologic study of the sections showed the results to be those of a double infection. And why not? From the nature of two such diseases, and from what is coming to light as we learn

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more of the nature of amebic infections, such cases should not be any more surprising than are other more or less frequent associations of infectious diseases.

Such double infections by amebas and other factors, particularly those which may produce ulceration in the colon, as typhoid fever, Bright's disease, etc., are not of infrequent occurrence; but this fact can no more be justly used as an argument to prove the secondary actions of amebas than can typhoid ulceration be said to be secondary to malaria, when these two diseases happen to exist together in the same patient.

A less tangible argument which is often used in support of this proposition, is based upon the supposition that amebas are frequently found in the normal intestine, where they do no harm until a "cold," "indigestion," "diarrhea," or what not causes changes in the colon, which enable the amebas to assume a pathogenic rôle. There is evidence that in some cases this is true, to the extent that such conditions may facilitate the propagation of parasites which happen to reach the bowel during such a time; but certainly there is little to indicate that it is more universally so with amebas than in the case of very many of our accepted etiologic agents.

Pathogenic and nonpathogenic amebas.—Since the appearance of the work of Quincke and Roos, Kruse and Pasquale, and others, who furnished evidence in support of their conclusion which appeared satisfactory at that time and which had a tendency to harmonize the two former extremes, many supporters of these ideas have attempted to discover simple means which could be used for diagnostic purposes, in order to distinguish pathogenic from nonpathogenic amebas. This has always been recognized as difficult; but, as it is usually applied at the present time, consists in recognition of certain supposed morphological differences between the parasites and in the unlike results obtained in animal experiments. In general, the larger amebas, some of which contain red blood cells, etc., have been considered pathogenic and to distinguish them they have usually been termed *Amebæ dysenteriæ*. The smaller species, which are considered nonpathogenic, do not contain red blood cells and show other morphologic variations in structure, are quite generally designated *Amebæ coli*.

Formerly, writers largely concurred in this opinion, but more extended experience has forced some changes in their views, if not with reference to the general question of pathogenicity and

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nonpathogenicity, certainly as to the value of all methods which have heretofore been applicable in distinguishing such differences. Long experience in the examination of stools and in post-mortem examinations have fully convinced us that the size and other morphologic appearances of the amebas have but little, if any, relation whatever to their pathogenesis.

Some of the most persistent and even fatal cases of dysentery may show amebas of not over 10 to 20 μ in diameter in stools during life and in the intestinal ulcers, post-mortem. On the other hand, only very large amebas may appear in some of the cases most amenable to treatment and in fatal cases which show the smallest amount of ulceration. Cultivation of the amebas in artificial media has demonstrated that the variation in size may be influenced at will by a number of conditions.

The value of the inclusion of red blood cells by amebas as a point in differential diagnosis is also exceedingly doubtful. There are many cases of amebiasis which run the regular course and at necropsy show the typical lesions, but in which inclusions of red blood cells by the amebas are not observed in repeated stool examinations during life. There is even evidence to show that the presence of such cells is not an indication of parasitic activity, but rather one of decay or degeneration. As has already been said, the young, active amebas in artificial media are quite select in their diet and among other substances avoid red blood cells, but as degeneration, resulting from toxic effects or other causes, sets in, less selectiveness in the choice of food is manifested and consequently whatever is within reach is taken up by the protoplasm.

The argument purporting to prove the existence of at least two varieties of amebas, based on the presence of harmless ones in the normal intestine and in those of persons suffering from other diseases, has already been discussed.

Another method which has been employed in facilitating a classification of these parasites consists in the results of animal experiments, which will be considered presently. When one regards the contradictory character of the results of experiments made with stools known to be dysenteric, it seems that we may justly conclude that to adduce further data of this nature as a basis for a classification of amebas is unwarranted.

This is true of the whole list of arguments heretofore given which have been used for purposes of differential diagnosis. Neither

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one nor all taken together are sufficient to justify the conclusion that there are two kinds of amebas found in the human intestine. *We do not wish to be understood as denying that there are pathogenic and nonpathogenic varieties of amebas; what we desire to say is that, thus far, the existence of such varieties has not been proved, nor are we as yet able to settle the question.* Still our work has been convincing along certain lines. Amebas cultivated from various sources, including the dysenteric intestine, the Manila water supply, lettuce, etc., have proved pathogenic under certain conditions, which reverses the view held of some of those formerly considered harmless.

All amebas are or may become pathogenic. This proposition, pending a complete solution of the problem, is the only safe one to adopt from the standpoint of public health in the Tropics. To admit such a proposition adds much to be explained by natural immunity and other conditions. This is especially true in a country where these parasites are found everywhere—in water, in earth, on vegetables, fruits, the skin, etc., and where, at most, an inconsiderable part of the population use any care whatever to avoid ingesting them, and it therefore must be true that they are taken daily into the gastro-intestinal tract by large numbers of persons. This being so, if all amebas are pathogenic, why have we not more amebic dysentery? And if all are not pathogenic, why do we not find them oftener in the normal intestine?

We have never followed a case which indicated that nonpathogenic amebas were propagating in the intestine, and we are sure that such an occurrence is very rare in the Philippine Islands, where, as has been said, the larger part of the inhabitants take in thousands of amebas daily. For we have had no difficulty in cultivating amebas from the very water they drink, and in producing dysentery in monkeys by the ingestion of cultures from this water under favorable circumstances. If, then, for example, this water contains both harmless and pathogenic amebas, the number of nonpathogenic ones, capable of resisting the stomach acids and of multiplying permanently in the intestine, are very few in comparison with the number of pathogenic ones, capable of resisting the same influences and found associated with the lesions of a pathologic entity.

We are not prepared to state that all cultures of amebas from water would give the same positive results as the one just men-

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tioned, but the question is now open to solution by the methods described in this paper. It is perfectly compatible with all the facts known about the infection to consider all amebas pathogenic, although the evidence now at hand does not warrant such a conclusion. The principal points in this connection have already been considered in the chapter devoted to the cultivation of amebas, and need not be repeated here.

Animal experiments.—Animal experiments with substances containing amebas, since Lösch's first case in 1875, have given conflicting results, owing to the necessarily crude methods of inoculation due to the absence of cultures, and these results have been used as evidence both for and against the pathogenic action of amebas. Before commencing a discussion of our work, it seems advisable to note briefly some of the results of others:

Lösch (1875) injected the stools of dysenteric patients into the rectum of four dogs, one of which had previously received an injection of croton oil. One of the dogs which had not received the croton oil developed symptoms of colitis.

Hlava (Uplavici) (1887) introduced dysenteric stools into the rectum of seventeen dogs, obtaining positive results in two cases. Six cats were similarly treated, with four positive results. Eight rabbits and six guinea pigs gave no symptoms of infection.

Kartulis (1889) injected stools containing amebas into the rectum of dogs, monkeys, guinea pigs, and rabbits, with negative results. Cahen (1891) had similar results with cats.

Calandrucio (1890) swallowed encysted amebas and at the end of twelve days found similar forms in his normal stools.

Kovacs (1892) introduced amebas into the cecum and rectum of dogs without obtaining any effects attributable to these parasites.

Quincke and Roos (1893) injected amebic dysenteric stools into eight cats, six of which died in from two to three weeks with amebic dysentery. Typical ulcerations containing motile amebas were found at necropsy.

Kruse and Pasquale (1893) produced dysentery in eight out of sixteen cats by rectal injections. Similar results were obtained with bacteria-free pus from liver abscesses.

Zancoral (1893) says that it is difficult to produce dysentery in animals by means of rectal injections. He succeeded, however, in giving rise to dysenteric symptoms in this manner—a result which he was also able to produce by the use of liver abscess pus free from amebas.

Roos (1894) injected dysenteric stools into the rectum of eight cats while they were under ether anesthesia. Six of these contracted fatal cases of dysentery, dying in from ten to twenty-two days with the presence of amebas in the intestinal contents.

Gasser (1895) produced dysenteric lesions in cats by means of rectal injections.

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Fijardo (1896) injected the contents of liver abscess and amebic stools into the rectum of cats. In no case was a typical infection produced, but one of the animals which had been treated with the feces died in four days without dysenteric symptoms or lesions at necropsy, and the other in twenty-four hours after passing bloody mucus stools containing amebas. At the post-mortem examination of the latter case no ulceration was observed, but the mucosa of the rectum was swollen and red.

Strong and Musgrave (1900) obtained positive results in several cats by the rectal injection of stools from dysenteric patients, and in one by the injection of culturally bacteria-free liver abscess pus. The post-mortem examination in these cases showed typical amebic ulceration.

Jäger (1901) made injections of similar stools into four cats, three of which were infected thereby, but the fourth of which showed no symptoms of the disease. One of the animals which died at the end of a month revealed typical amebic ulceration at necropsy.

Ucke (1902) infected two young cats by rectal injections of dysenteric stools. Both of the animals died of inanition. At necropsy neither ulcerations nor amebas were found in the intestinal canal, although the organisms were present in the stools during life.

Huber (1903) infected dogs by feeding them stools containing encysted amebas.

Harris (1901) produced dysentery and liver abscesses in puppies by rectal injections of feces containing amebas, but when mixed culture of all the bacteria which grew on plates from similar stools were used in a similar manner, no symptoms were produced. He therefore concluded that amebas were the etiolic factors in this disease.

Cunningham, Grassi, Calandrucio, and others maintain that the great resistance of amebas in the encysted stage may play a significant part in the transmission of infection from one animal to another. This is borne out by the experiments of Quincke and Roos (1893), who fed two cats with stools containing amebas, which had been allowed to encyst by leaving them at room temperature for from two to nine days after being passed. Both of these animals developed dysentery, showing the presence of amebas in the stools and at necropsy amebic ulceration of the colon. In similar experiments with motile amebas no infection resulted.

As to the other side of the question, there have been a large number who have failed to obtain positive results on repeating the experiments here enumerated. At any rate, at present, as Janowski expresses it, there is nothing more to be gained by this sort of work.

It seems that those who previously believed in the pathogenic rôle of amebas have already become convinced of the reliability of many of these experiments, while those who do not accept the etiologic rôle of these parasites will never give up so patent an argument as is offered by the necessary crudeness of all experiments made with stools or liver abscess contents.

It seems to us that the sum total of this work, particularly that part in which positive results were obtained by the injection of

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amebic pus culturally free from bacteria, is all but convincing, but in order to furnish sufficient proof of this fact to the unsatisfied, it will be necessary to adopt other methods of experimentation, and the natural method is that of culture. Such experiments have apparently heretofore not been made with any great frequency.

Kartulis (1891) asserted that he had produced dysentery in cats by rectal injections of pure cultures of amebas isolated from a liver abscess as well as with impure cultures of amebas obtained from the dysenteric intestine and grown upon his straw-infusion medium. Feeding similar cultures gave no results. Kartulis also produced temporary diarrhea in two dogs by rectal injections of amebas grown together with bacteria in sterilized rabbit dung.

Vivaldi (1893) injected cultures of amebas, which he had grown in straw infusion, into the rectia of cats, and diarrhea followed. At necropsy catarrh of the colon was observed, but there was no evidence of ulceration.

Casagrandi and Barbagallo (1897) conclude that amebas which have been grown on artificial media are not parasitic, because such cultures do not produce the disease in cats.

Zaubitzer failed to obtain pathogenic action with cultures of his straw-infusion amebas. After feeding guinea pigs with these cultures, he was unable to find the parasites in the stools. But when the experiments were repeated in frogs, encysted amebas were found in the stools and recovered by culture. He thoroughly lavaged the bowels of cats and then introduced cultures and stitched the anus, but obtained negative results.

Fiori fed cultures of the *amœba coli* of Celli and Fiocca, isolated from the water of Alexandria, to a patient suffering with chronic diarrhea. which at intervals was dysentery. Amebas were found in the stools for a few days, but the course of the disease was not altered.

Kartulis finally injected straw-infusion cultures of what he considered to be dysenteric amebas into the rectum of a cat and closed the anus by suture. On the sixth day there were mucus stools. The animal died of dysentery, but no necropsy was performed.

Our experiments with cultures of amebas on animals, a number of which are briefly described below, have been quite convincing as to the etiologic rôle of some amebas. These results have been particularly satisfactory with monkeys, but nothing definite has been gained by working with cats, dogs, and other animals. Even with monkeys, infections have not been constant, and the lesions produced in them, while satisfactory, have not been so extensive as those usually seen at necropsy in man. Otherwise the specimens obtained have had the macroscopic appearance of amebic infection and the parasites have always appeared in the contents of their intestines. A study of sections from the colon has confirmed the nature of the infection in each instance.

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It will be noticed in looking over the brief protocols that most of our animals were killed rather than allowed to die. This was done in order to secure fresh necropsies when it appeared that an animal would not live until the following day. Just why the experimental disease when once established, should run so rapid a course in monkeys, we do not know; but it corresponds in every particular with the cases of natural infection which have come under our observation in the same class of animals.

During a recent epidemic of pneumonia among the inmates of Bilibid Prison we were able to secure an abundance of material particularly showing the very early lesions of amebiasis; and these lesions so closely resemble many of those seen in our experimental animals that it makes us all the more certain of our results in the latter. We do not wish at this time to go into the pathology of this infection, much of which remains to be described. The classical lesions of advanced cases are well recorded, and some notes on earlier ones have been carefully made by Councilman and Lafleur, Harris, Diamond, and others; *but there is undoubtedly a somewhat definite preulcerative pathology and varying stages and varieties of early ulceration, as well as a greater variety of the more advanced lesions than has generally been recognized.* The materials which we now have would indicate that both the macroscopic and the microscopic pictures show variations as great as those observed in the intestinal lesions of typhoid fever, including the nonulcerative type.

It will also be noticed in looking over the attached protocols, that the incubation period in monkeys is a rather long one, and in those animals which gave satisfactory results from a single feeding it is remarkably constant. This is borne out by one human infection and offers still further evidence of the latency often seen in the naturally contracted cases. The symptoms in experimental cases in monkeys closely resemble those observed in man. Anemia and emaciation are constant, and are manifested before the development of diarrhea. In some of these the onset is rather sudden with severe diarrhea, which continues almost unabated until death, but as in the case of man, it more often starts as an intermittent diarrhea, which becomes more severe with each exacerbation.

In most of our experiments the animals have been under observation during from ten to thirty days. A large number of monkeys,

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including those not inoculated and the ones used for other experiments, have been constantly kept in the same room with our animals under identical surroundings, and of more than one hundred of these, and of probably two hundred and fifty which have been passed through the animal yards at the Serum Laboratory, only three have been found attacked with spontaneous amebic infection, two of these developed in animals kept in cages beside those in which our experimental animals were confined.

We have occasionally observed cases of naturally contracted amebiasis in monkeys at other times, but such instances are not frequent, and so far as we have been able to trace, occur only in animals which for some time have been in captivity and have thus had opportunities similar to those of human beings to contract the infection from sources which to them are unnatural. At any rate, considering the care which we have taken to see that our experimental animals were free from infection at the time of feeding, natural infection could hardly be entered as criticism of our work. Neither could it be said that monkeys are natural hosts of amebas, for these parasites are not often encountered in their stools, and the lesions found in the naturally contracted cases are in every particular like those of the experimental ones.

There is one point in these experiments which indicates that a lowered vitality may have to do with the certainty and the intensity of the infection in the individuals exposed to it naturally. When animals which have been fed amebas are injected intra-abdominally with cultures of bacteria which of themselves would cause illness without death, the incubation period of the amebic infection has been considerably shortened.

Some of our monkeys which have been fed amebas, and which have developed diarrhea with the presence of the parasites in their stools, have recovered. This is, no doubt, occasionally true in the naturally contracted disease in man.[1]

[1] Owing to the extremely chronic character of many of these infections, characterized as they are by long periods of quiescence between exacerbations, it is difficult to find the earliest moment when recovery is complete. We have, however, been convinced that such is occasionally the case even in the *unacclimated* Caucasian, and more often so in the native, because we have observed such recoveries in one Caucasian and three natives, in all of which the diagnosis was unquestionable, treatment was never instituted, and more than a year has elapsed since there was clinical evidence of the disease, or amebas in the stools.

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We have not succeeded in infecting a monkey with young cultures of motile amebas, though in some of the experiments the ones used were but a few days old and cyst formation hardly complete.

Some interesting facts have come to light regarding the rôle of the symbiotic bacteria which are present in the mixed cultures used in our experiments. Attempts to reclaim these bacteria from stools of the infected animals have not been made in all cases, but in some instances such bacteria fed have not been isolated, indicating that the symbiosis had been changed in the intestine of the animal. This was not found to be true, however, in the case of man. The yellow, pigment-producing bacillus in symbiosis with the amebas in the culture ingested by the subject was recovered from the stool after symptoms of dysentery had developed.

Monkey No. 536[1] had been under observation in the laboratory for fifteen days, during which time diarrhea was not present and amebas were not found in the stool by microscopic examination.

On February 9, 1904, it was given by stomach tube 10 cubic centimeters of a distilled water emulsion of old encysted cultures of Ameba 11524 in symbiosis with *Spr. cholerœ*. No further treatment of any kind was administered, and the animal apparently remained well until the twenty-seventh day after feeding, when a slight diarrhea was noted. By the thirty-third day this diarrhea had become very decided; there was considerable mucus, a few red blood cells, and fair numbers of amebas in the stools. Stained preparations and peptone cultures gave no evidence of the presence of *Spr. cholerœ*. From the thirty-third to the thirty-seventh days the diarrhea abated somewhat, but by the fortieth day had become a moderate clinical dysentery, which progressed until the forty-seventh, when the animal was killed.

At necropsy, which was performed immediately, emaciation and anemia were marked. The walls of the colon were thickened, the mesocolic glands slightly enlarged, the subserous vessels injected, and the omentum adherent to the cecum. The colon contained fluid material with mucus and blood, and showed a hemorrhagic catarrh of the mucosa throughout. Ulcers from 2 to 5 millimeters in diameter were found scattered throughout the mucous membrane of the colon. These ulcers were rather superficial in character and contained numerous amebas, some of which enclosed from one to three red blood cells. Cholera spirilla were not found. The amebas microscopically, resembled those which were fed and were not reclaimed by culture. (See fig. 3, Pl. I.)

A monkey similar to the one above mentioned was fed repeatedly large doses of *Spr. cholerœ* of the same stem as that used in the preceding culture by Dr. W. B. Wherry, but without the production of diarrhea.

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[1] The monkeys used in these experiments were of the two varieties native to the Island of Luzon, *Macacus cynamolgus* and *Macacus philippinensis*.

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Monkey No. 442, on October 20, 1903, was fed 10 cubic centimeters of an emulsion of an old encysted culture of Ameba 11524. On October 22 this feeding was repeated. The animal remained well until November 21, when it was given in the abdominal cavity 5 cubic centimeters of an emulsion similar to that above mentioned. On December 2 it was injected with 10 cubic centimeters in the abdominal cavity, and on December 21, 20 cubic centimeters. In the latter part of December there was diarrhea for a few days. On January 6 diarrhea again developed, the stools containing mucus, a few red blood cells, and fair numbers of amebas. By January 22 the animal had become very much emaciated and anemic, passing frequent bloody stools, which contained large numbers of amebas.

On this date chloroform was administered and the necropsy performed immediately. There was marked emaciation and anemia. The inguinal and mesenteric glands were slightly enlarged. Old chronic peritonitis was evident, the omentum being firmly adherent to the intestines, the surfaces of which also adhered to one another in many places. The large intestine showed hemorrhagic catarrh throughout. Motile amebas, some of them containing red blood cells (one of them contained as many as eight), were found in the rectum and the cecum. The walls of the colon were thickened. The solitary follicles were enlarged and capped in places with superficial necroses. There were a few small superficial ulcers, measuring from 2 to 5 millimeters in diameter, in the rectum and the cecum. The anatomic picture in general did not differ from that seen in man when death occurs during the early stages of the disease. Amebas were not obtained in cultures from the colon.

Monkey No. 464, on November 18, was given subcutaneously 4 cubic centimeters of an emulsion of an old encysted culture of Ameba 11524; on November 24, 10 cubic centimeters of a similar emulsion; and on December 7, 20 cubic centimeters. On December 9 the abscesses which had formed at the points of the injections were opened and found to contain numerous amebas, which grew on culture. On December 12 the serum from this animal showed no parasiticidal action on the amebas cultivated from the abscess. On December 21 another subcutaneous injection of 15 cubic centimeters was given. On the 24th diarrhea developed. From January 1 to 7 the dejecta were dysenteric in character and quite numerous.

On January 12 the animal was chloroformed and necropsy performed at once. Emaciation and anemia were marked. The large intestine contained a considerable amount of mucus and a small amount of blood, and on microscopic examination showed large motile amebas. The walls of this bowel were thickened, and the mucous membrane showed hemorrhagic catarrh. The solitary follicles were slightly swollen, and there were a few small superficial ulcers. Amebas were not obtained by culture from the colon. We believe this animal infected itself from the abscess by scratching and licking the wounds and the fingers.

Monkey No. 678 was a healthy animal which had been under observation for ten days, during which no amebas were found in the stools. On May 2, 6, and 9 increasing doses of *Spr. choleræ* were injected subcutaneously. On May 11 a "pure mixed culture" of ameba 11524 and *Spr. choleræ* was

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injected into the right lobe of the liver. On May 15 the animal was chloroformed and necropsy performed immediately.

The liver appeared normal, except for slight hyperemia at the point where the inoculation needle entered. Neither amebas nor cholera spirilla were found in this organ. The mucosa of the colon was observed to be congested, and there were small hemorrhages into some of the solitary follicles. A few of the latter showed superficial necroses with the presence of fair numbers of amebas.

Monkey No. 371, on September 16, was given, by high enema, 5 cubic centimeters of an emulsion of an old encysted culture of Ameba 11147. One month later the animal developed a slight diarrhea, and at necropsy, performed immediately after death by chloroform, a few amebas were found in the contents of the colon. There was considerable mucus in the cecum and the ascending colon, but no blood and no macroscopic lesions were evident.

Monkey No. 493, on December 12, was given in the abdominal cavity 5 cubic centimeters of an old encysted culture of Ameba 11147. Another injection of 15 cubic centimeters was made on December 21. On January 6 the animal was killed, and no lesions were found at necropsy.

Monkey No. 555, on February 23, was fed 5 cubic centimeters of an emulsion of an old encysted culture of Ameba 11147 together with *Spr. choleræ.* On March 14 diarrhea developed, and on the 17th the animal died with clinical symptoms of dysentery.

At necropsy there was marked emaciation and anemia. There was catarrh of the colon throughout, this being most marked in the cecum and the ascending colon, where there were a very few small ulcers. The liver contained several small abscesses, measuring from 1 to 3 millimeters in diameter, which enclosed bodies resembling amebas, but which were not motile. The animal had been dead eight hours at the time of necropsy.

Monkey No. 479, on November 30, was given subcutaneously 2 cubic centimeters of an emulsion of Ameba 11147. On December 2 the animal died of dyspnea. At necropsy there was a small amount of dirty viscid pus at the point of inoculation, which, however, contained no amebas microscopically. There was an abscess about 2 millimeters in diameter in the left lung, which contained numerous motile amebas. The colon was normal and no other significant changes were found in the body. Amebas were not obtained by culture from the contents of the lung abscesses.

Monkey No. 553 had been under observation for one month, and during this time had shown no diarrhea, and its stools contained no amebas. On February 23, it was fed 10 cubic centimeters of an old culture of Ameba 25624. The animal died on April 5, after a diarrhea of two weeks' duration. Necropsy was performed ten hours post-mortem. Emaciation and anemia were very marked. The liver and heart showed fatty degeneration. There was slight general lymphatic enlargement. The colon contained fluid material, with considerable mucus and some blood. The mucosa throughout the colon was very much swollen and in a few places showed superficial necroses. Amebas similar to the ones which had been fed were present in the stools during life, but on account of delay in necropsy the contents of the colon were not examined post-mortem.

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Monkey No. 554 had been under observation for twelve days, and during this time had shown no diarrhea and stools contained no amebas. On February 22 it was fed 10 cubic centimeters of an old encysted culture of Ameba "water." About one month later diarrhea developed, and the animal became very much emaciated. On March 26 it was killed by chloroform. At necropsy, which was performed immediately, the walls of the colon were found to be thickened. There were several small ulcers in the cecum as well as a sloughing of the mucous membrane over a considerable area near the splenic flexure. Numerous amebas, some of them enclosing red blood cells, were found in the contents of the bowel. (See fig. 2, Pl. I)

Monkey No. 526, on January 7, was chloroformed and the cecum exposed, after which 10 cubic centimeters of an old culture of Ameba "water" was injected from a hypodermic syringe into this organ. On February 4 a violent diarrhea developed, and the stools contained numerous amebas, some of which enclosed red blood cells. The animal was killed by chloroform and necropsy performed immediately. The mesocolon was slightly enlarged and pale. The mesenteric and intestinal blood vessels were very much injected, and there were adhesions around the operation wound (which healed by first intention). The colon contained semifluid feces with some mucus and blood. The cecum splenic flexures showed marked hemorrhagic catarrh. The solitary follicles were swollen throughout, some of them showing early necrosis, and others, beginning small ulcers 1 to 3 centimeters in diameter. These little ulcers contained large numbers of amebas, which were also found throughout the intestinal contents. Amebas grew out in culture from the ulcers of the colon.

Monkey No. 538, on February 10, was given in the abdominal cavity 10 cubic centimeters of an old culture of Ameba "water," which had been grown from monkey No 526. By February 25, a large abscess had developed at the point of inoculation, continuing to increase in size until March 8, when the animal was killed. The abscess contained thick, yellowish pus, and bodies resembling encysted amebas; but there were no motile parasites. The colon was normal and free from amebas.

Monkey No. 537, on February 10, was inoculated in the liver with 1 cubic centimeter of a suspension of an old culture of Ameba "water" obtained by culture from the colon of monkey No. 526. On February 16 the animal was killed and necropsy performed immediately. The under surface of the diaphragm was much congested and adherent to the dome of the liver over an abscess about 1 centimeter in diameter. This abscess was quite superficially situated and had the gross appearance of an amebic abscess of the human liver. Microscopically the pus was found to contain bacteria and amebas, some of which enclosed red blood cells. The colon was normal and free from amebas. Amebas were not grown by culture from the liver abscess.

Monkey No. 542, on February 16, was inoculated into the stomach with cultures from the liver abscess of monkey No. 537. This abscess had been produced by cultures of amebas from the stools of monkey No. 526, which had been in turn infected by feeding with old cultures of Ameba "water." On February 23, the animal was killed. There were numerous actively

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motile amebas in the cecum, but no macroscopic lesions were found in the colon, and no attempt was made to reclaim these amebas by culture.

Monkey No. 518, after being proved free from infection, on December 29, 1903, was inoculated subcutaneously with 10 cubic centimeters of an old culture of Ameba "water." On January 2 an abscess formed, and on January 22, diarrhea developed with large motile amebas in the stools and continued until January 27, when the animal was killed. Lesions of an early amebic infection were present.

One of the ulcers 6 millimeters in diameter, was situated on the ileocecal valve, and another one, a little smaller, in the cecum. Amebas were present in the ulcers and were obtained by culture. As with the two other monkeys already mentioned, it is believed that this animal infected itself from the abscess, a rational explanation to those familiar with the action of these animals under such conditions.

Monkey No. 517, on December 29, was inoculated in the abdominal cavity with 15 centimeters of Ameba "water." On January 3 the abscess which had formed at the point of inoculation ruptured, and its contents were found to contain amebas. The animal was killed and the intestinal canal was found to be free from amebas and normal in appearance.

Monkey No. 534.—On March 24, 2 cubic centimeters of an old culture of Ameba "lettuce" was injected into the liver along with a bacillus from the city water supply. On April 10 the animal was killed. At necropsy the intestine was found to be normal. There were two liver abscesses, the larger one being 10 millimeters in diameter. On section these abscesses had the gross appearance of an amebic abscess of the human liver. Amebas and bacteria were obtained by culture from the contents of one of the abscesses.

Monkey No. 552, after being proved free from clinical symptoms and amebas, was inoculated on February 23, in the stomach with the aid of a catheter, with 6 cubic centimeters of an old culture of Ameba "lettuce." On March 14, diarrhea developed, but no amebas were found. On March 17, the stools contained considerable mucus and blood, and amebas were present in large numbers. On March 19 the animal was killed. Necropsy showed hemorrhagic catarrh of the entire colon, which contained a large amount of bloody mucus in which were found numerous amebas. There was a general thickening of the walls of the colon, and numerous small superficial ulcers were present. (See fig. 1, Pl. I.)

Monkey No. 531, on January 27, was inoculated subcutaneously with 1 cubic centimeter of a stool containing large numbers of amebas. An abscess developed. The animal was killed on February 9. The intestine was found to be normal. The subcutaneous abscess contained amebas, which were reclaimed by culture.

Monkey No. 357 died from spontaneous diarrhea on August 30, 1903. At necropsy there was found to be catarrh of the colon, but no ulceration. No amebas were obtained by culture.

Monkey No. 558, which had been kept in a cage adjoining experimental animals, spontaneously developed profuse diarrhea on March 25. The stools contained numerous amebas. Death occurred from dysentery on March 26. At necropsy there was found tuberculosis of the lungs and

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and spleen. The colon showed hemorrhagic catarrh, and contained numerous small ulcers, which enclosed amebas.

Dog No. 441, on October 20, was fed old encysted cultures of Ameba 11524. This treatment was repeated on October 22 and 26 and November 9 and 11. There was no diarrhea, and no amebas were found in the stools up to this time. On November 12 the dog was given in the abdominal cavity, 10 cubic centimeters of an emulsion of a similar ameba in melted agar. The animal died on November 24, of acute general peritonitis. No amebas and no lesions were present in the colon.

Dog No. 553, on January 29, was given by stomach tube 10 cubic centimeters of an emulsion of an old encysted culture of Ameba 11524. There was no diarrhea, but anorrhexia, anemia, and emaciation developed early and were progressive until the animal died on March 14. At necropsy, which was performed ten hours post-mortem, there was marked catarrh of the entire gastro-intestinal canal, but there was no ulceration, and no amebas were found in the contents of the colon.

Cat No. 366, on September 4, was fed 5 cubic centimeters of an old encysted culture of Ameba 11147. This treatment was repeated on September 7, 9, and 15. The animal remained well until October 19. On this date and on November 24 additional treatments were administered; but there occurred no symptoms of bowel trouble up to December 4, when the animal was killed. At necropsy no amebas were found in the intestinal contents, and no macroscopic lesions were present.

Cat No. 363, on September 1, was fed 5 cubic centimeters of a twenty-four-hour bouillon culture of Bacillus 9650b. This treatment was repeated on the second and third. On the fourth 5 cubic centimeters of an emulsion of an old culture of Ameba 11147 and of Bacillus 9650b was introduced into the stomach by tube. This treatment was repeated on September 7, 9, and 15. On October 15 diarrhea developed, and on the nineteenth the stools contained considerable mucus and some blood. The animal was chloroformed on the twentieth and necropsy performed immediately. Aside from moderate anemia and emaciation, there were no lesions of importance except in the colon, which contained a considerable quantity of mucus, motile amebas, and some blood, as well as numerous small punctate hemorrhages. There was some swelling of the solitary follicles with superficial necrosis, but no distinct ulceration.

Cat No. 350, on October 20, was given by high enema 5 cubic centimeters of a young growing culture of Ameba "water." This treatment was repeated on October 25, 28, and 31 and November 2. No diarrhea or other symptoms were produced, but the animal was killed on November 4, and necropsy was performed at once. The lower portion of the colon appeared normal, but the cecum contained a considerable amount of semiliquid fecal matter and mucus. There were no macroscopic lesions of the mucosa, and no amebas were found.

Cat No. 353 was given 5 cubic centimeters of an old culture of Ameba "water" by high rectal tube. This treatment was repeated on alternate days until five injections had been given. During the next month there were slight attacks of diarrhea with some emaciation; but no amebas were

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found in the stools. At necropsy the cecum contained some mucus and some blood, but no parasites were found. The walls of the colon were thickened, without other decided evidences of infection.

Cat No. 349.—The large bowel of this animal was cleaned with glycerin enemas, followed by lavage with warm water. After one hour 5 cubic centimeters of an old culture of Ameba "water" was introduced by means of a catheter high up into the colon, and a similar injection was thereafter given every four days for two weeks. No symptoms of dysentery were produced. At autopsy, made two months after the first injection, no lesions and no amebas were found in the colon.

A healthy man, kept under observation for ten days, during which time his stools were not found to contain amebas either microscopically or culturally, on repeated examinations following cathartic doses of Rochelle salts, ingested three ordinary gelatin capsules, which contained scrapings from three-week-old cultures of Ameba 11524 in symbiosis with a bacterium which had been proved harmless for animals and man.

On the twelfth day after treatment there was slight diarrhea, and amebas was the first time present on microscopic examination. From this time until the twentieth day there was mild intermittent diarrhea. On this day some tenesmus was complained of and the stool contained considerable mucus and a small amount of blood in addition to large numbers of amebas, some of which enclosed red blood cells. This evidence was considered sufficient to establish the diagnosis, and the patient was placed under treatment. Both the ameba and the bacillus introduced were reclaimed by culture on the day after the development of diarrhea.

IV. CONCLUDING REMARKS.

The term "amebiasis," which has been introduced in this article, denoting an infection with amebas, is comparable in its application to filariasis, trypanosomiasis, uncinariasis, etc. It is not open to the objections so frequently offered to "amebic dysentery," "amebic enteritis," and the other names usually given to the disease.

Amebas, when present in water, soil, and other places outside the animal body, may almost constantly be secured in culture, and by the methods described in this paper pure species in pure cultures of bacteria may be obtained. Such pure species of amebas may be secured by comparatively simple means, and their isolation with pure cultures of bacteria—the "pure mixed culture" of Frosch—by an equally practical technique.

Amebas may be cultivated from dysenteric stools and from ulcers in the human bowel. This has been done in 2 per cent of the cases by the direct inoculation of the material containing the amebas on the surface of the media recommended. Positive results are more frequently obtained (30 per cent) when the media are

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simultaneously treated with certain bacteria. They may be grown in a still larger per cent of cases (60) when the media are smeared with the bacteria isolated from the same source as the amebas.

Living bacteria or other microörganisms seem to be necessary to the existence of the amebas under artificial condition.

Amebas show a certain selectiveness for symbiotic bacteria, and this selectiveness may be increased and changed on artificial media.

The passage of cultivated amebas through the animal organism, either subcutaneously or by the gastro-intestinal canal, increases the selectiveness of the parasite for symbiotic organisms and the difficulty of its growth on artificial media. This phenomenon appears to be progressive with successive animal inoculations, until in some cases the cultivation becomes impossible. If these injections are made into the liver of monkeys, the progressive difficulty of growth increases rapidly with subsequent animals, and the cultivation on artificial media soon becomes impossible with the means at command.

There is as yet no way of determining beforehand what bacteria will furnish satisfactory symbiosis; but the variety of those which may act satisfactorily with amebas in the human intestine is probably large and includes many of the ordinary and well-known organisms, both pathogenic and nonpathogenic. The same one, however, is not satisfactory for all amebas.

Amebas have not as yet been grown in pure culture. Whenever they have been freed from other microörganisms by various methods, they have refused to multiply on any known medium.

There are certain differences in the resistance of different cultures of amebas to certain physical and chemical influences.

The cultivation of pure species of amebas has offered strong evidence of the plurality of species of these protozoa, and this plurality apparently extends to those which produce infection in man.

The transient appearance of amebas in the normal intestine is a much rarer occurrence than it is generally believed to be, and their persistence in this location without the production of lesions for a time greater than that of the known latency of some cases of infection has not been shown.

Other evidence brought forward to show the harmlessness of amebas is not conclusive, and certainly in the Tropics the

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appearance of amebas in the stools should be sufficiently diagnostic for the institution of therapeutic measures regardless of the nature of the clinical symptoms.

Infection in the colon of monkeys follows the feeding of amebas under certain described conditions, and such pathogenic ones may be cultivated from a number of sources.

In one instance amebas cultivated from the dysenteric intestine with a certain bacillus produced clinical amebiasis by ingestion in man; while the cultures of this bacillus alone failed to produce such symptoms.

Amebas are the etiologic factors in the disease generally known as amebic dysentery, and by following the methods described in this paper, such amebas may be grown on artificial media, and the disease reproduced in monkeys and man by the ingestion of these cultures. Amebas may be reclaimed by culture from the stools or the intestinal ulcers.

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EXPLANATION OF ILLUSTRATIONS.

PLATE I. Illustrating the appearance and early lesions of experimental amebiasis in monkeys.[1]

Fig. 1. Section from the colon of monkey No. 552 which was fed 6 cubic centimeters of an emulsion of Ameba "lettuce." Diarrhea developed on the nineteenth day and the animal was killed on the sixth day thereafter. Drawing by T. Espinosa.

2. Section from the colon of monkey No. 554 which was fed 10 cubic centimeters of an emulsion of Ameba "water." Diarrhea developed on the thirtieth day and the animal was killed on the fourth day thereafter. Drawing by T. Espinosa.

3. Section from the colon of monkey No. 536 which was fed 10 cubic centimeters of an emulsion of Ameba 11524. Diarrhea developed on the twenty-seventh day and the animal was killed on the twentieth day thereafter. Drawing by Dr. W. B. Wherry.

FIGS. 1, 2. Plate cultures, illustrating the method of securing cultures of amebas with pure cultures of bacteria, Photograph by Martin.

3. Showing the spread of amebas and bacteria on plate cultures. The amebas are seen as white spots in the margins and points of the more homogeneous bacterial growth. The peculiar character of the spread of the combined growth is due to the wanderings of the amebas and the growth of the bacteria carried along by them. See page 29. Photomicrograph by Martin from a twenty-four-hour-old plate culture of Ameba 11524, in symbiosis with *B. coli*. (Zeiss objective AA, ocular No. 4. Bellows, 30 centimeters.)

4. A forty-eight-hour-old plate culture of amebas, illustrating the distribution of amebas on the plate in the margins of the growth where the bacteria are not numerous. Encysted forms as well as those in all stages of motion are shown. Photomicrograph by Martin. (Zeiss objective AA, ocular No. 4. Bellows, 30 centimeters.)

5. An eight-day-old plate culture of amebas, showing the "agglomeration" of amebas sometimes seen in cultures. The amebas are all in the "round stage." Photomicrograph by Martin. (Zeiss objective AA, ocular No. 4. Bellows, 30 centimeters.)

[1] For a full description of the experiments see the text on pages 70–76.

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FIG. 6. A three-day-old culture of amebas, showing their rapid multiplication in two colonies of air bacteria which developed in the plates after thirty-six hours. This ameba was afterwards isolated in pure culture with the bacteria shown. Photomicrograph by Martin. (Zeiss objective AA, ocular No. 4. Bellows, 30 centimeters.

7. A photomicrograph of a cover-glass impression from a twenty-four-hour-old plate culture of Ameba 11524 stained by the Wright-Romanowsky method for blood. The contractile vacuole contains a bacterium. Photomicrograph by Martin. (X about 900.)

8. A cover-glass impression from two twenty-four-hour-old plate culture of Ameba 11524 stained by the Wright-Romanowsky method for blood. Photomicrograph by Martin. (X about 900.)

9. A cover-glass impression from a twenty-four-hour-old plate culture of Ameba 11524 in symbiosis with B. 12935. Stained by the Wright-Romanowsky method for blood. The nucleus and the so-called spores and vacuoles are distinctly shown and a granular appearance of some of the so-called spores is also well shown. Photomicrograph by Martin. (X 2000.)

10. A cover-glass impression from a twenty-four-hour-old plate culture of Ameba 11524, stained by the Wright-Romanowsky method for blood. The nucleus, vacuoles (?), spores (?), and and bacteria are shown. Photomicrograph by Martin. (X 2000.)

11. A sterile-water emulsion of Ameba 11524 from a twelve-hour-old plate. Unstained preparation. Photomicrograph by Martin, instantaneous exposure. (X 800.)

12. Ameba water. Manner of preparation and amplification of the same as in fig. 11. Photomicrograph by Martin.

13. A section from a monkey's intestine, showing the distribution of amebas in the preulcerative lesions. Photomicrograph by Martin. (X about 500.)

14, 15, 16. Cover-glass impressions from twenty-four-hour-old plate cultures of Ameba 11147 stained by the Wright-Romanowsky method for blood. Fig. 14 illustrates the rather dense ectoplasm often noticed in unstained specimens of this ameba. Figs. 15 and 16 show forms somewhat suggesting conjugation. Photomicrographs by Martin. (X about 900.)

17. Cover-glass impression from a forty-eight-hour-old plate culture of Ameba 25624 stained by the Wright-Romanowsky method for blood. The "agglomeration" and distribution of chromatin is well shown. Small masses of chromatin are also shown outside the amebas. Photomicrograph by Martin. (X about 850.)

18. A cover-glass impression from a thirty-six-hour-old plate culture of ameba lettuce stained by the Wright-Romanowsky method for blood. Photomicrograph by Martin. (X about 850.)

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FIGS. 19. Cover-glass impression from a twenty-four-hour-old plate culture of ameba lettuce stained by the Wright-Romanowsky method for blood. Photomicrograph by Martin. (X about 900.)

20. A cover-glass impression from a three-day-old plate culture of Ameba "G" stained by the Wright-Romanowsky method for blood. Photomicrograph by Martin. (X about 800.)

21. Fresh distilled-water preparation from a six-month-old culture of ameba water in symbiosis with a pure bacterial culture. Photomicrograph by Martin. (X about 700.)

22. Fresh distilled-water preparation from a twenty-four-hour-old culture of Ameba 25624 stained *in vivo* by a dilute solution of neutral red, and photographed after all the parasites have assumed the "round stage." Photomicrograph by Martin. (X about 700.)

23. Distilled-water preparation from a six-month-old culture of Ameba 11147, showing the radiations formed by the bacteria along the cyst walls. Photomicrograph by Martin. (X about 650.)

24, 25. Distilled-water preparations of Ameba 11524 from young cultures. Stained *in vivo* by dilute solutions of neutral red, and photographed after all the parasites have become round or encysted. Photomicrograph by Martin. (X about 900.)

26–32. Illustrating various pictures of encysted forms of Ameba 11524, which, taken consecutively, indicate very strongly one cycle in the life of this parasite.

Fig. 26 illustrates a one-month-old culture containing round forms and both young and mature cysts.

Fig. 27 is from a six-month-old culture (same as fig. 26), showing old cysts.

Fig. 28 is from a three-hour-old transplant from the preceding culture (fig. 27), showing the filling out, nuclear prominence, and other early changes preparatory to further multiplication.

Fig. 29 is also from the preceding culture five hours after transplantation to fresh medium.

Figs. 30, 31, and 32 are from the same transplant at a still later time, and show more advanced formation of the young amebas and a gradual thinning of the cyst wall on the side which, in fig. 32, has reached a point where rupture of the cyst wall is complete.

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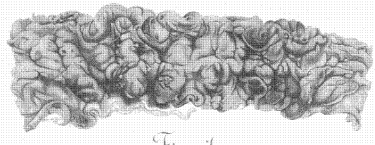

Fig. 1

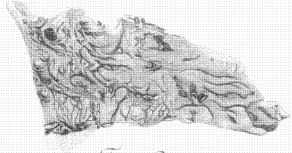

Fig. 2

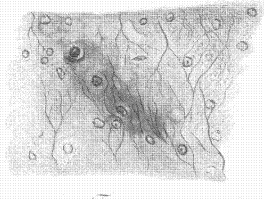

Fig. 3

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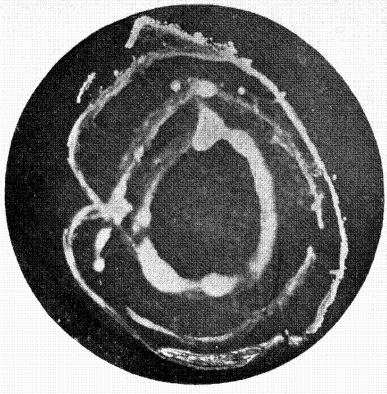

Fig. 1.

Fig. 2.

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FIG. 3.

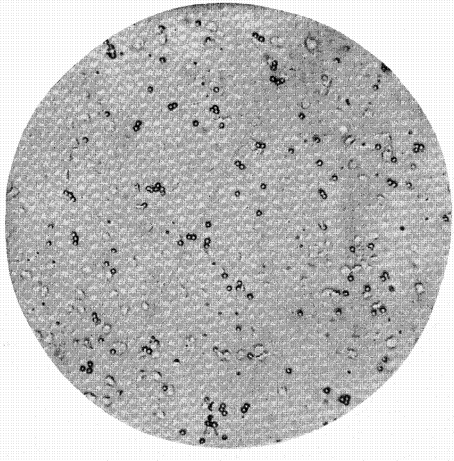

FIG. 4.

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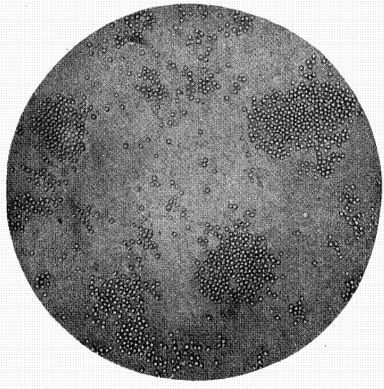

FIG. 5.

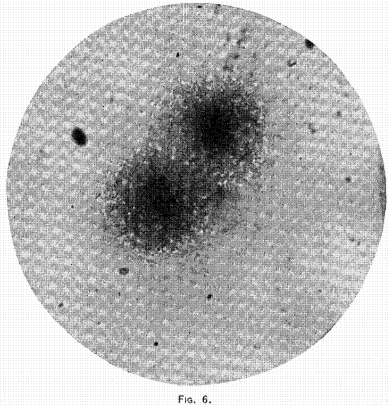

FIG. 6.

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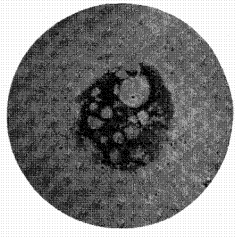

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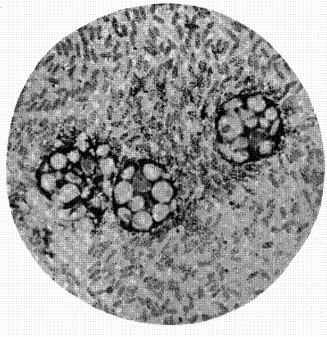

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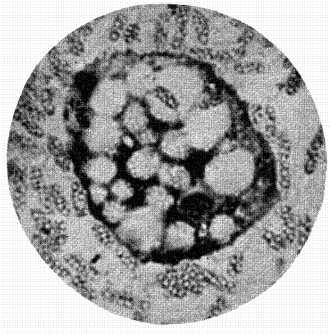

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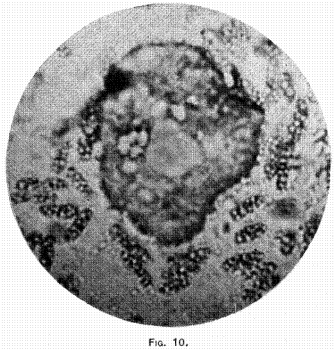

Fig. 10.

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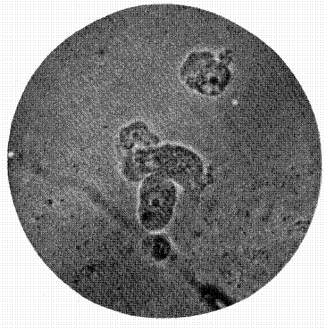

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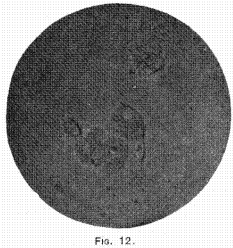

Fig. 12.

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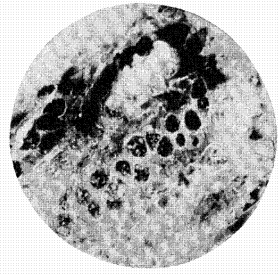
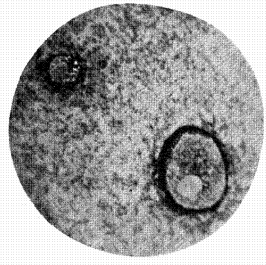


Fig. 13.



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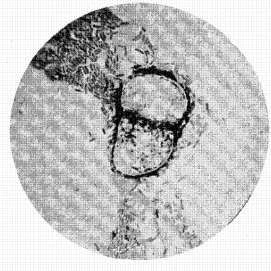

Fig. 15.

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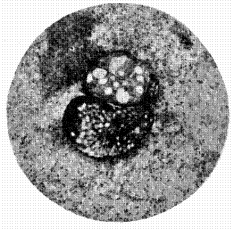

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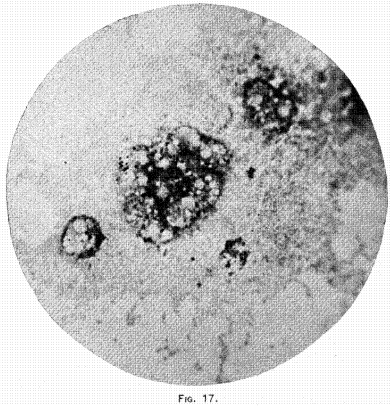

Fig. 17.

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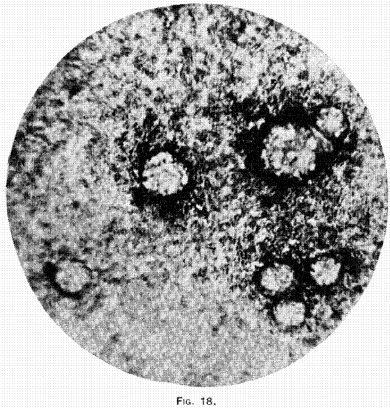

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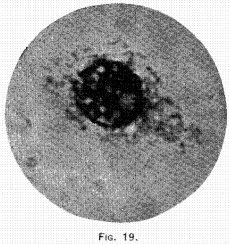

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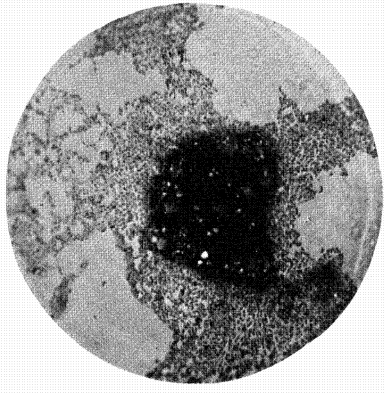
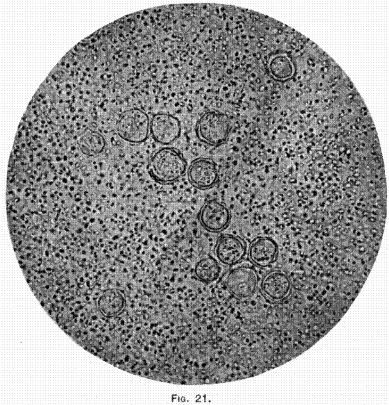


Fig. 20.



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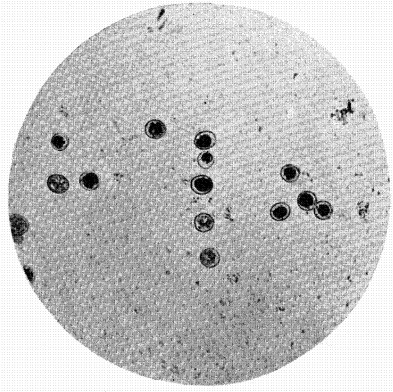

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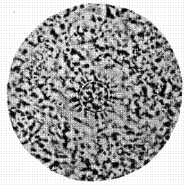

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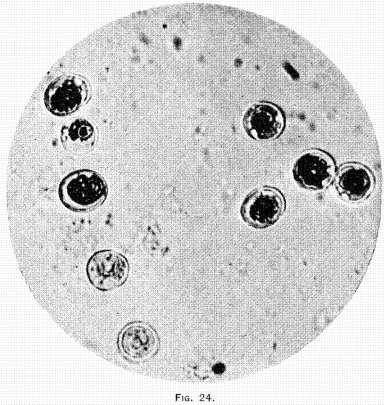

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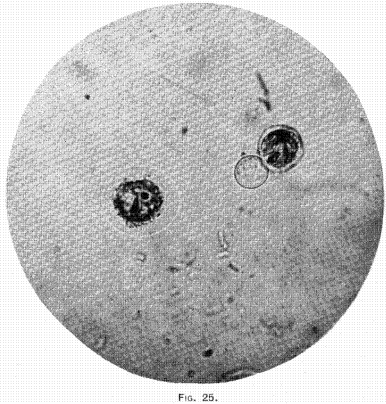

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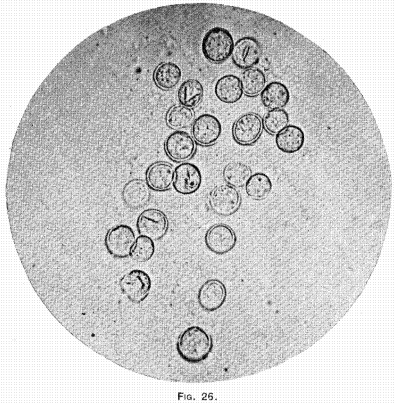

Fig. 26.

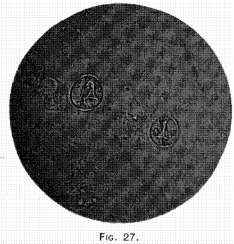

Fig. 27.

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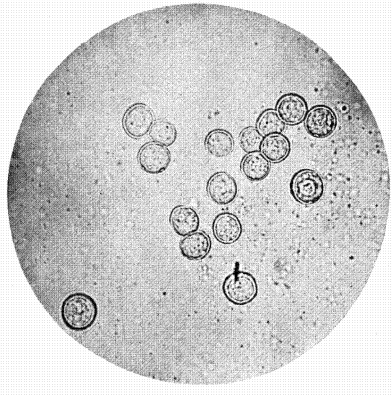

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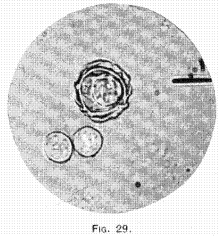

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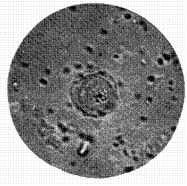

Fig. 30.

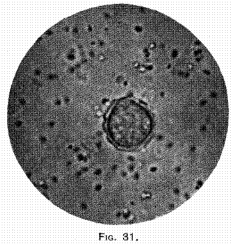

Fig. 31.

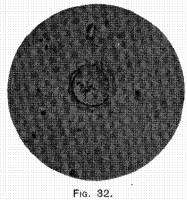

Fig. 32.

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PART II.

TREATMENT OF INTESTINAL AMEBIASIS (AMEBIC DYSENTERY) IN THE TROPICS.

By W. E. MUSGRAVE, M. D.

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SYNOPSIS.

I. Introduction.
II. Prophylaxis.
    (*a*) Water for drinking purposes, kitchen and pantry, toilet and bath; ices and ice water.
    (*b*) Vegetables and fruits.
    (*c*) Soils.
    (*d*) Personal factors.
    (*e*) Public health.
III. Treatment.
    (*a*) General remarks.
    (*b*) Diet.
    (*c*) Change of climate.
    (*d*) Drugs and chemicals.
    (*e*) Local.
IV. Appendicial amebiasis (amebic appendicitis).
    Pathology; classification.
    Symptomatology; treatment.

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PART II.

TREATMENT OF INTESTINAL AMEBIASIS (AMEBIC DYSENTERY) IN THE TROPICS.

By W. E. MUSGRAVE, M. D.

I. INTRODUCTION.

The happiest results which may follow any research are those which lend themselves to their practical application in the prevention or cure of disease, and nearly all of the great advances made in therapeutics are of this class.

Because the progress made in the treatment of intestinal amebiasis since its identification as an independent disease by Osler, Councilman, and Lafleur has been of an empirical nature, there has been a long delay in introducing the methods most generally employed at the present time, and these are still far from satisfactory. The differences which exist regarding the treatment of the disease in various places within the geographic belt where it is endemic, have also, to a certain extent, been brought about by the question as to the rôle which the amebas play in its causation. Heretofore it has not been possible to be certain in regard to this because of the failure to cultivate the parasite, but now that this is made a practicable procedure and the etiologic rôle of amebas is established, the first essential to building up a rational therapeutics is fulfilled.

Formerly, owing to failure to cultivate amebas and therefore without definite knowledge of the distribution of pathogenic ones in nature, rules for prevention have been formulated without

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specific data in regard to this particular disease, and the result has been a greater lack of uniformity and success in the use of prophylactic measures than in the treatment of the infection. Inefficacy on one hand, and unnecessary severity on the other, have most certainly been the result of this ignorance.

In Part I of this report some of the important questions relating to prevention and cure of amebiasis have been elucidated, and methods and lines of work opened which will undoubtedly furnish other important data. However, this work is not complete, and a full discussion of methods of prevention and treatment, according to our present knowledge, will probably necessitate changes in the near future regarding some questions which are as yet indefinite or unexplained.

Therefore this paper will deal with the more important and heretofore neglected points in prophylaxis and treatment, and an attempt will be made to utilize some of the experimental facts which have been brought out in Part I of this report.

The complication, amebic appendicitis, is really a continuation of the disease from the cecum to the appendix, and for that reason, and because of its importance in considering therapeutic measures, it will also be briefly discussed.

Intestinal amebiasis (amebic dysentery) is the medical subject of the most universal interest in the Philippine Islands. It causes more than 50 per cent of the invalidism of public servants; it must be reckoned with in the profit-and-loss accounts of business enterprises, and it enters into consideration in military movements. It numbers among its victims all ages, all classes, and all races, being most prevalent in Manila among the unacclimated adult Americans.

Theoretically, at least, it is a preventable disease, but just how far the various theories which are supposed to insure prevention are of practicable and necessary application, while still allowing some of the comforts of life, is the problem to be solved for successful prophylaxis against it. Heretofore the only recommendation based upon facts which could be made was that of the ideal prophylaxis which, briefly expressed, is *to take nothing into the gastro-intestinal canal which has not been sterilized by heat.*

Such a procedure, while efficient, is not always practicable, is always inconvenient, and as we learn more of the habitat and manner of life of the amebas, we find that it is not necessary in

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every respect. However, it is so nearly so that all departures from it should be in the nature of specific exceptions. Previously all such exceptions were in a sense due to speculation, and their variety is responsible for many cases of the disease. There are numerous Americans who have taken advantage of less stringent measures and who have escaped the disease during four of five years' residence in Manila. Others who have apparently followed similar recommendations have fallen victims, and finally, there are numbers of foreign residents in Manila to-day who have been more than four years in the country who have not contracted dysentery and who have eaten and drunk without the slightest regard for the consequences.

The principle of natural immunity (used in its most inclusive sense) is very noticeable in this disease, but it depends upon so many conditions, it is often transient, and finally, as it can not be determined by justifiable means, any conclusions based upon observations such as are given above must be unsatisfactory. However, with methods of cultivation of amebas at our command, this problem of the simplest "technique of living" compatible with safety is a practicable one.

II. PROPHYLAXIS.

To fully grasp the importance of preventive measures it must be remembered that amebic dysentery is endemic in the Philippine Islands, and that amebas which can not be differentiated from the infecting agent are easily isolated from a surprisingly great variety of substances.

Water furnishes a prolific source of the organisms, and the majority of cases of the disease can be either directly or indirectly traced to it. Practically, all the surface waters of the Philippine Islands contain the parasites in large numbers, and all the cisterns and wells which we have examined are even more richly supplied with these and other parasites. Rain water stored in cisterns is perhaps the most dangerous.

The city water supply of Manila contains large numbers of protozoa; and amebas, not distinguishable from those found in the intestinal ulcers in dysentery, may be drawn from the tap water almost at any time, and as shown in Part I of this report, cultures of some of these amebas when ingested by monkeys produce dysentery in these animals. The danger from water has become quite

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generally recognized by the public, and honest effort to overcome the difficulty is used, but the methods looking to this end are very often ill advised.

First to be considered is the use of distilled water. Much of the so-called distilled water in the city is either not distilled at all or it is stored in reservoirs which are not properly cared for, and, consequently, amebas and other parasites may be frequently cultivated from it. On the other hand, it often happens that a safe water is contaminated by handling after its receipt from a reputable source. It is taken into a vessel which has been washed in tap water, or, if this is not the case, is placed on ice in bottles which have had similar treatment. In the last few weeks we (Mr. Clegg) have cultivated amebas from the water kept in bottles for drinking purposes from five different residences, and similar results have been obtained in cultures from the water coolers in public offices. Where water is boiled for drinking purposes, similar contaminations may result from handling, and filtration should never be relied upon for preparing drinking water unless the filter and method of its use and care are thoroughly understood. The rather extensive practice of filtering water after it has been boiled is particularly pernicious. If filtration is desirable it should always be done first and the water boiled afterwards.

The use of carbonated and other bottled waters, both imported and of home manufacture, is coming more and more into general use, and but for their expense would soon solve the problem for the better class of people. Here, though, as with the distilled waters, care in the source of the supply is necessary, for many of the local bottling works issue a product of doubtful purity, and in some instances have sold such an article under the label of a more reputable firm. Such observations must also be extended to imported waters, for some of those which are at times on the market here with labels of waters known to be pure are (in reality) bottled on some of our neighboring coasts and are certainly, in certain instances, dangerous. However, perfectly safe and good bottled waters, both domestic and imported, are on the market.

The question of the judicious use of water in the kitchen and pantry is an important one, although satisfactory recommendations are difficult. In the Tropics the housewife does not feel inclined to spend time in the kitchen, and servants are not trustworthy. The average kitchen in Manila is dirty, and is supplied

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with tap water which usually is used by the servants in preparing foods and cleaning all dishes and vessels. From what has already been said, the danger from such sources is obvious, and the correction is practicable and not very expensive. Every kitchen range could well be supplied with a large hot-water tank, such as is commonly used in America, and this tank should be the only source of water for both kitchen and pantry. An ordinance requiring the installation of such a boiler as a part of the plumbing system of houses of the better class would do something toward controlling a disease which causes more invalidism than some of the other more prominent infectious diseases for the control of which large sums are annually spent by our Government.

Water used for the mouth should, of course, receive the same care as that for drinking purposes. The city water is satisfactory for the bath, provided care is exercised to avoid its introduction into the mouth or rectum. Such water, of course, should never be used either for the vaginal douche or as a simple enema. Both these practices are suprisingly common and many cases of dysentery are undoubtedly brought about by the use of the latter.

In Part I we (Mr. Clegg) have shown that amebas are much more dangerous when in the encysted stage, and that, among other causes, cold will always cause the parasites to assume this condition. From this it is obvious that water, as well as other articles of diet which contain amebas, is more apt to cause infection when taken cold. Of the substances served in this way, the ices and ice cream deserve especial notice. It is perfectly certain that much of the "sorbete" sold on the streets contains encysted amebas, and careful analysis would probably show the impurity of some ices from more reputable sources. These are standard preparations for social functions, where they are served in large quantities under abnormal conditions, and hence increased elements of danger of infection are always perfectly apparent. After one of the large receptions given last autumn we cultivated dysenteric amebas from the washings from the ice-cream dishes and from the water in which they were washed, while samples of the ice cream taken from the original container were free from them.

The surfaces of uncooked green vegetables and fruits are another frequent habitat of amebas and a common means of the transmission of the parasite to the human intestine. Since the foreign population has become educated to the point where they use drink-

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ing water of a quality which is, as a rule, safe, the use of green vegetables is probably one of the most fertile of the remaining sources of infection, and the number of people, otherwise reasonably careful, who will eat these things with impunity without any special preparation, or at most, after they have been rinsed in distilled water, is perfectly surprising. Others who would not risk such a course will allow their use in garnishing foods, or in flavoring soups, which is accompanied by almost as great a risk. Lettuce, one of the most frequently infected of vegetables, was formerly not used by the more intelligent class of people, but recently it has become possible to obtain a nice-looking quality of this article at the Agricultural Experiment Farm, and it has become quite a popular article of diet.

On February 9 we (Mr. Clegg) obtained some lettuce from this farm with a view of determining the presence or absence of amebas, both before and after washing the leaves. One head was placed in a large sterile flask, a quantity of distilled water added, and the whole thoroughly shaken for several minutes. The water was then carefully drained into another sterile flask, and, without removing the lettuce from the original container, it was submitted to four additional washings, any one of which was more thorough than would be given by a careful housewife. Tubes of fluid from each of these washings were then centrifuged, and a microscopic examination showed amebas in two of them. Cultures were made from all these flasks and amebas which, as has been shown in Part I, were those of true amebic dysentery, grew in every tube, although they were most numerous in the first washing.

Nearly all other vegetables and some fruits yield results similar to that which we obtained with lettuce; the fruits with thick, sound skins and those which are strongly acid are *probably* safe articles of diet, but it must not be forgotten that one of the first amebas ever cultivated was from sour, fermenting grapes. Boiling water kills amebas almost instantly, and there are but few fruits or vegetables which are damaged materially by scalding, provided they are first made very cold by placing them for a time on ice.

Soil is a natural habitat of amebas, and the organisms in such a location are particularly numerous in the Philippine Islands. From such a source water which might otherwise contain but few amebas is often richly supplied. This is particularly shown by the almost epidemic spread of the disease following the recent

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flooding of Manila and which brought with it the washing out of cesspools and other places where otherwise the parasites might have remained harmless. Methods of personal cleanliness and other necessary precautions to avoid the introduction of amebas from such sources into the alimentary canal suggest themselves.

Alcohol, as in may other diseases, has received a great deal of discussion as a preventive agent in dysentery. It undoubtedly exerts a destructive action, even in moderately dilute solution, but that its habitual use is of value in preventing infection is not proved. The acidity of the normal stomach probably plays an important preventive rôle, and, while hyperacidity may follow the use of alcohol, that condition is not necessarily constant.

There are certain important personal factors which enter into a consideration of prophylaxis. Children are less susceptible to the disease, and in them it is more amenable to treatment. This is particularly fortunate when their habits and the difficulty with which they are controlled are considered. The unacclimated Caucasian is the most liable to contract amebiasis, and the newcomer should always secure proper advice in order to escape preventable consequences.

One should strive by exercise and, in general, by living a well-regulated life, to keep in good physical condition; lowered vitality from any cause, diarrhea, indigestion, constipation, and many other personal factors certainly predispose to the infection.

Amebas, not distinguishable from those in the intestinal contents in amebic dysentery, have been found present in the mouths of healthy people, and it behooves residents of the Tropics to give scrupulous care to these exposed mucous membranes by frequent cleansing with distilled water or some mild antiseptic.

The normal acidity of the stomach is probably one of the preventives to the entrance of living amebas into the alkaline intestine, and its derangement, which is so frequent in warm countries, is increased materially by fermentation and other abnormal conditions in the mouth. The hands are so often in contact with materials containing amebas, and in many instances are used in the preparation and serving of food, that their cleanliness always should be a consideration. Above all, *methods of prevention must not be used spasmodically.* Safety depends upon constant and consistent adherence to a well-defined plan of living, and no

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departures from it, even in minute details, should be made without securing the approval of competent authority. Among the better classes, the theoretical outline of living is generally very good, but a violation in the details of its consistent execution is extremely common and flagrant. Examples of this might be cited almost indefinitely, and the manner of infection is more often traced to such lapses in daily practice rather than to a fault with the general plan of living.

The medical profession is partly to blame for all these things. Our first duties are to guard the public health, and in the Philippine Islands there is certainly no other one thing so important as to teach the public a proper technique of living.

Finally, I wish again to emphasize that amebiasis is an infectious disease, and to point out the desirability of judicious municipal ordinances and regulations looking to its control. I hardly need call attention to the fact that its ravages do more to cripple Government and private enterprise of all kinds and conduce more to personal dissatisfaction with the country than do those of all other infectious diseases combined.

The control of cases of amebic dysentery by public-health officers is neither necessary nor desirable, but regulations requiring the report of every case with certain data would accomplish good in several ways. Valuable facts for future work could be obtained, and, what is more important, such a procedure would particularly call forth public attention and interest and would prepare public opinion for coöperation and support in controlling the disease.

For several years the military have been improving regulations looking particularly to the prevention of this disease, and results of these precautions are shown in the following figures:[1] During the month of July 439 patients drawn from approximately 2,742 troops, were treated at the First Reserve Hospital, and of this number 9 were suffering with amebiasis.

During the same month 318 patients from approximately 2,500 civil employees were treated at the Civil Hospital and by private physicians, and of this number 90 were suffering from amebiasis.

[1]The figures used here have been furnished by Maj. J. M. Bannister, surgeon, U. S. A., commanding officer First Reserve Hospital, Manila, by Dr. H. E. Stafford, Attending Physician and Surgeon, civil officers and employees, and by a number of other physicians in Manila. My thanks are due to these gentlemen for their courtesy.

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During the month of July, therefore, over 3 per cent of 2,500 civil employees suffered from amebic dysentery while but three-tenths per cent of 2,742 soldiers were treated for the same trouble.

There are business houses here whose employees live according to sanitary rules laid down by a physician, and such a practice should extend to civil employees and be more general among the public.

A civil employee arriving here from the United States gains his ideas of how to live from lay sources, and in the military he receives his information from published regulations. Provision by which employees of the Civil Government could receive proper instruction in the elements of prevention would amply repay such efforts by increased efficiency, just as it has proven to be the case in both the military and in private enterprises.

III. TREATMENT.

General.—Successful attempts in the classification of dysenteries from an etiologic standpoint are so recent that the great majority of the literature relating to the treatment is of but little value in its application to intestinal amebiasis (amebic dysentery), which is now a generally recognized etiologic clinical and pathologic entity—a disease different from other forms of intestinal flux in many essential particulars.

In general, it may be said that one form of treatment, in Manila at least, is now quite commonly in use, but the tendency is too much to routine. I wish to emphasize in the beginning that *any routine treatment* of this disease will prove unsatisfactory in a large number of cases. There is no disease with which we have to deal which requires more careful study of individual cases and greater variation, within limits, in the maner of applying remedial measures.

Before instituting treatment of any kind the patient should have a careful physical examination, and the history should include, particularly, efforts to specifically locate the manner of infection. Palpation of the abdomen, in connection with the history, will usually furnish considerable evidence of the duration of the disease, and what is more important, the location of the lesions in the bowel. A weekly record of the weight will always be of service, and is one of the best indications of progress.

The treatment of the patient should be governed somewhat by the findings made at the physical examination, by the clinical mani-

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festations, which are so varied in this infection, and by the probable duration of the disease at the time the patient comes under observation.

Acute dysenteric manifestations, whether they come on early in the disease (which is rare) or as an exacerbation in cases of some standing (which is frequent), require the same general measures as those applicable to acute colitis from other causes. The patient should be confined to the bed, and should be required to use the bed pan; the diet should be fluid, in the form of peptonized milk, pressed meat juice, whites of eggs beaten up with soda water, lime juice and soda, etc. The pain may be controlled by local applications or by opium which can be given internally in the form of Dover's powder or paregoric, or subcutaneously as morphine. Calomel and salines, and sedative or cold-water enemas are also useful.

After the subsidence of the acute symptoms and in all other cases, the general measures should be much less severe. It rarely is necessary or even desirable to confine patients to the bed, and in the great majority of cases, in the absence of decided dysenteric manifestations or symptoms of some complication, carefully directed exercise exerts a most beneficial influence.

It would be found a good rule to confine all patients to their rooms during the first few days of the treatment because of some of its disagreeable features, and exposure to the hot sun is, of course, to be avoided at all times. In the early evenings and early mornings, however, a drive will often accomplish more good than many doses of tonic medication, and where patients have comfortable home surroundings it will rarely be necessary or advisable to send them to a hospital.

Where emaciation and enemia are advanced, particular attention must be paid to building up the general health, and with well-nourished patients, treated early in the disease, care must be used to maintain this condition during the more or less lengthy and trying period of active local treatment.

Diet.—Notwithstanding the possible presence of extensive ulceration, the appetite and digestion often remain good, and when we consider the local nature of the disease and that, excepting complications, death frequently results from asthenia, it is readily seen how urgently necessary is a sufficient amount of nutrition.

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The tendency, at least in Manila, has been to unnecessarily limit the diet in the ordinary type of cases, but the greatest reason for any such restriction is to limit the mass of fecal matter in the colon, where it may interfere somewhat with the efficacy of local treatment. When the stomach and small intestine are deranged, and sometimes when this is not the case but where clinical manifestations show involvement of the cecum or upper colon, a selected diet is indicated; and when the rectum is very irritable such a restriction quite frequently appears to increase the tolerance for the enemas. But, in the more usual type of cases, there is little evidence that a liberal diet interferes in any way with the efficacy of the local treatment—in fact, quite the contrary often may be observed. In some of the more advanced infections, where emaciation and anemia are progressive, and where, as is often the case, the patient has no desire for the usual liquids, etc., a carefully prepared beefsteak will be relished and borne with comfort. The amount of residue in such instances does not need to be considered, for it may be disposed of by a simple enema given a half hour before the usual local medication, a precedure which, in any event, is often useful.

It has come to be my custom in cases of the ordinary type, and in all where severe clinical manifestations have subsided, to allow a very liberal diet; forbidding only irritating foods and such as are likely to ferment and those leaving a large bowel residue. In the class of cases where the cecum alone or the cecum and ascending colon are involved, the clinical manifestations are often very slight, and extensive ulceration may be present before the nature of the disease is suspected. Treatment of these is difficult because of the care necessary to reach the lesions by enemas and because of the soft fecal matter usually clinging to the mucous membrane in this part of the bowel. A fluid diet sometimes makes a material difference in the treatment, but even here an occasional saline cathartic or a large simple enema once or twice daily will usually accomplish more and at less expense to the patient's strength than a reduction in the amount of food.

Change of climate is an important factor in treatment. Alone, it is not a specific therapeutic agent in any sense of the word, but it is an especially valuable aid, particularly in old, emaciated cases, and should be employed, where possible, with all patients who do not react to treatment in local environment. However, except

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under extreme necessity, it should never be recommended nor patients allowed to pass from observation without first having a course of local treatment, and great caution should be exercised in advising a change to those who have symptoms of some of the more common complications. In nearly all cases the improvement is but temporary, unless treatment is continued, and for that reason patients should have specific directions to consult a physician upon arrival at their destination, and, where practicable, letters should be given explaining the condition.

Drugs.—The list of drugs which have been used in dysentery and vaunted from time to time, many of them as specific, is too long even to mention, and, so far as they may have any *specific* curative properties in the type of the disease which is under discussion, they are all useless.

It requires no very extensive observation to show that this practice of drug giving is a much-abused one, and the actual harm done by it is not inconsiderable. There was a time in this country when such things were excusable, but with the knowledge obtained by experience and research thir continuation as routine treatment deserves the severest condemnation.

There are a few drugs which, on account of their very extensive use, deserve special consideration. First, the various salts of bismuth, particularly the subnitrate and subgallate, are both useful therapeutic agents within limits, but their abuse in the treatment of amebiasis is very great. In the absence of the more rational local treatment, in combination with some of the other internal remedies, bismuth may be a very useful drug, and at other times is probably very nearly harmless, but when given at the same time quinine enemas are being used it undoubtedly does do harm.

Observations made at necropsy furnished abundance of evidence in support of this statement. As is well known, these salts are insoluable in the intestinal canal, and so their action is largely mechanical. In a bowel which has been ulcerated for some time, they impregnate the edges of the ulcers and all other more or less dead tissue to such an extent that at post-mortem, even after the most thorough washing, enough is often left to make the surface almost black. The coating formed is so tenacious and fastens itself so firmly about the ulcers that quinine and other curative substances applied locally have little opportunity to reach the most essential places. There is also ample clinical evidence

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corroborating these statements. I have seen patients who have taken bismuth internally together with quinine enemas for considerable periods of time, while amebas were constantly present in the stools but, on substituting small doses of a saline for the bismuth, a permanent disappearance of amebas occurred within a comparatively short time. The more usual and equally tenable arguments against the indiscriminate use of this drug need not be entered into. The important consideration which may be amply demonstrated is that the drug is not harmless, as it is popularly believed to be, but, during the time of local treatment, it is capable of doing much damage in a negative way if in no other.

Much of the abundant literature about the use of ipecac is valueless because of the lack of sufficient clearness as to the type of the disease referred to by the writers who advocate the drug. Whatever may be its value in certain other forms of dysentery, it is quite certain that when given in the doses and the manner prescribed by its most ardent adherents, it is not only useless, but may be dangerous in amebic infections of long standing. In small doses it undoubtedly acts as a tonic to mucus surfaces, if nothing more, and as such often may be administered with advantage in intestinal flux. In emaciated patients who have had the disease for a long time, and when given in the large doses which are usually recommended, its results are sometimes disastrous. I have seen three cases (with post-mortem) in which, in my opinion, it was the immediate cause of death. These were patients who should have remained alive for weeks, unless complications had developed, and one of them might have recovered under more rational therapy.

Magnesium sulphate and other salines have been much used in the treatment of dysentery, and where a cathartic is indicated in well-nourished patients they are very satisfactory, but their routine use for considerable periods of time, particularly in patients in the more advanced stages of the disease, is not to be recommended. They have, at least, two specific actions—one is to increase the alkalinity of the intestinal contents and thus favor the propagation of amebas; the other is to cleanse the mucus membrane and thereby allow greater efficiency of the enema. Further than when indicated as an active cathartic for this latter action, they should be used in this disease with caution.

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There is some reason for the use of the mineral acids, especially hydrochloric and nitro-muriatic, and the efficiency of hydrochloric acid is increased almost always by its combination with pepsin; sometimes other digestive ferments are useful. In the vast majority of the usual cases, the acid-pepsin solution has at least two favorable actions—first, that due to its acidity alone, and second, it is particularly active in reducing or preventing the nausea which is often a troublesome sequence to enemas, which condition is principally due to reversed peristalsis. For this purpose neither the pepsin nor the acid alone is as satisfactory as the combination.

The so-called intestinal antiseptics are often of service in allaying fermentation, and possibly may also limit somewhat the number of bacteria. Salol or guiacol carbonate in combination with minute doses of ipecac often exert a good influence, and the more recent preparation—acetozone—gives good results as an adjuvant in many cases. To get the best results from acetozone it should be drunk freely in 1–5,000 to 1–2,000 solution, as much as one to three or more liters being consumed in twenty-four hours. It is much more palatable when the solution is made in carbonated water, and one of the most satisfactory methods of prescribing it is to have it carbonated in syphons or ordinary soda bottles with directions to use instead of water. Its action as an intestinal anteseptic is, like other preparations, somewhat limited, but in cases where there is active fermentation of the stomach and upper bowel it often gives good results. I have seen some unfortunate results follow the administration of celloidin-coated capsules of this drug, probably due to rupture in the intestine of imperfect capsules and the consequent liberation of the chemical in concentrated form. Its use in enemas will be considered under the local treatment.

Strychnin and other powerful stimulents should be used with care during the administration of enemas on account of their stimulating action in the bowel. Strychnin, particularly, is a valuable general tonic and stimulant, but during the employment of local treatment it is best replaced by some of the more diffusible drugs; alcohol in the form of champagne, dry sherry, or punches, is usually grateful to the patient and satisfactory in results.

Except in those cases complicated with malaria the internal administration of quinine so highly recommended by some is use-

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less, and even when malaria *is* present the objects sought are much better obtained by the quinine enemas which, if properly administered, will result in cinchonization of the patient. Indeed, this is one of the disagreeable features of this treatment in a very large percentage of cases.

Calomel has had may advocates on account of its supposed antiseptic action in the intestine, and given in small frequent doses it may exert a beneficial influence.

I have never seen satisfactory results from the use of sulphur. In fact, as stated before, there is no internal medication which is in the least sense specific in intestinal amebiasis, and such treatment should be directed to the improvement of the general health of the patient and to the alleviation of some of the prominent symptoms as they arise. All insoluble substances which coat the bowel are contraindicated during the time that the patient is being treated by enemas.

Local treatment, properly carried out, gives satisfactory results in the largest number of cases, but to insure success constant care, close study of individual cases, and the surmounting of many obstacles are necessary.

The manner of giving the enema determines in no slight degree its efficiency. Routine is particularly dangerous, each case requiring careful consideration, and variations should be made according to the indications to be met.

The *apparatus,* in private practice, should consist of a glass irrigator of at least two liters capacity, inclosed in a metal frame, and in hospitals the larger adjustable glass irrigators meet the requirements particularly well. Rubber bags are never satisfactory for reasons obvious to those of experience in giving these enemas. The tube should be of very soft rubber five or six feet long and connected by a valve stopcock with the rectal tube, which in turn should be at least 100 centimeters in length, from 10 to 15 millimeters in diameter, and made of the best red rubber. It should be of moderate firmness, not so stiff as to be dangerous to the ulcerated bowel nor so soft as to easily fold upon itself during introduction.

Contrary to what is often recommended, the opening should be in the end of the tube, well rounded, and the immediate extremity slightly contracted and hardened. The disadvantage of having the opening in the end has been said to be that it is

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much more likely to become closed by fecal matter or pressure; but, in my experience, this has not been the case. Such a rectal tube will occasionally become clogged, but very rarely so when judgment is used in its introduction. The weakest point of this tube is its extreme end, and if any obstruction occurs during its entrance with the current flowing (as it should be under low pressure) it is quickly observed by a lack of pulsation in the soft delivery tube (which should always be under control of the operator's fingers), or by watching the fall of fluid in the irrigating tank, and such trouble always may be quickly corrected by withdrawing the tube very slightly and waiting a few seconds until the fluid, which should be allowed to escape in a pulsating manner from the delivery tube by alternating compression and relaxation, has removed the obstruction by dilatation of the bowel. If the tube opens both in the end and on the side the means of detecting an occlusion are the same as with the other. However, on account of the two openings, when the fall of fluid indicates a stoppage the tube already may have been folded. This trouble usually begins at the eye, and several feet of loop may have been introduced before both openings are closed and the flow is stopped. In this case a correction of the trouble necessitates a further withdrawal and the turning of a knuckle of an inch or more of tube in the ulcerated bowel. Finally, with the tube opening at the end there is obviously much less danger of mechanical injury to any deep ulcers which may be present. The tube with an opening on the side alone is, of course, not to be considered.

A very good position for the patient during the administration of an enema, and one which is usually most convenient, is the Sims position, with the hips well elevated. When practicable, it is still better to have the foot of the bed raised from 12 to 18 inches. The knee-chest position on the same kind of a bed is sometimes particularly satisfactory, both because of the ease in passing the tube and the ability to retain the fluid. With the rectal tube and anus well lubricated with clean castile or other mild soap or soft white vaseline, the tube, freed from air, is introduced through the sphincter. After a slight rest for the spasm of the sphincter to subside, the flow may be turned on under low pressure (controlled by the fingers on the delivery tube) and the latter is then slowly introduced to a distance of from 40 centimeters to 1 meter or more, depending upon the character of the case, the location of the

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lesions, and the patient's tolerance for the fluid. After the tube has been introduced to the highest point which is necessary the rate of flow may be increased and the amount of fluid to be introduced is governed by the bowel capacity or the patient's ability to bear the pain, which in some cases is excruciating.

Patients often will be found who will take from 3 to 4 liters with no great discomfort, but in the majority about 2 liters will be all which can be borne, and in some, not over a liter is practicable. Naturally this variation in quantity depends upon the differences in bowel capacity, upon its irritability, and upon the patient's idiosyncracy to pain. Women, as a rule, are more satisfactory patients in this respect than men.

Several other factors help in introducing a large quantity of fluid into the bowel, but they can not be determined always without experiment. Bowel spasm comes on in nearly all cases during the entrance of the fluid. In some instances increased pressure gives the best results, while in others a decrease in, or a temporary stopping of the flow, will be found most satisfactory. Often allowing a patient to change position during the flow will give relief, and if this is done with caution there is no objection to it. Occasionally a bowel is so irritable as to preclude the giving of an enema of sufficient concentration and quantity. This difficulty may be greatly relieved by giving one of warm water or salt solution containing one-fourth to one-half grain of morphin a half hour before the medicated enema is introduced.

The presence of gas in the bowel always increases the pain caused by the enema, and hence, in those cases where there is considerable fermentation or where the bowel has a large accumulation of feces, it is often well to give a preliminary simple one. The latter cause little or no pain, they cleanse the bowel, and in that way allow increased certainty of action of the medicine to follow. Their use gives such happy results in many cases and their inconvenience is so small that I have employed them very frequently as routine, even where there are no special considerations to be met.

After a full enema has been administered, a change of position usually enables the patient to retain it longer, and in addition may insure its reaching all parts of the colon. If the sloping bed has been used it will usually suffice for the patient to turn on the back with legs flexed, or on the right side, and if further

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elevation is desired it can best be accomplished by still further raising the foot of the couch.

In every case all practical methods should be applied to give the largest quantity of fluid which can be administered and retained, for to secure satisfactory results the diseased part of the bowel, which in the majority of cases is clinically the entire colon, must be distended and the fluid retained from five to fifteen minutes. Retention of the enema for less than five minutes hardly allows it to develop its full parasiticidal action, and when the fluid is held for more than fifteen minutes the result may be unnecessary inconveniences from the absorption of quinine. The latter is a very troublesome condition in very many cases.

The temperature of the solution not infrequently makes a material difference in its tolerance. It has been shown that the amebas when they are in the moving stage are most susceptible to the action of antiparasiticidal agents. This condition is interfered with by either cold or heat. For this reason cold solutions would appear at a glance, at least theoretically, contraindicated either as a vehicle for the curative medicine or as sedative applications immediately preceding the medicinal ones.

There is, however, another factor to be considered here, for while the cold increases the parasite's resistance to medicinal agents, it also, by causing it to encyst, removes its power of independent motion and its ability to cling to its surroundings and therefore it may be much easier to flush from the bowel.

Tuttle has recently reported good results in the treatment of amebic dysentery by the use of simple ice water enemas given frequently, and his success may be explained by mechanical removal of the parasite.

Cold solutions, while quite disagreeable during their introduction, later often have such a soothing influence on the mucus membrane and as a consequence for some time, when other remedies fail to create tolerance in the bowel, I have often used an ice-water injection one-half hour before the quinine enema, and sometimes have administered the quinine itself in cold solution.

After Tuttle's article more attention has been paid to this point. However, it must be remembered that the lowered temperature produced by cold injections in the bowel is only temporary, and that those amebas which are not flushed out during this time are not destroyed, and their progress is but slightly delayed.

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In order to secure whatever advantage might be attributed to the cold and at the same time to introduce the antiparasiticidal agent, a few old cases which were very resistent to treatment were placed on ice-cold solutions of quinine instead of the usual ones at the body temperature. This method has been used with about twenty persons in private practice, mostly among those who have not reacted well to the usual treatment. The results so far, while encouraging, have not been universally satisfactory.

Such ice-cold quinine enemas are often quite painful when they are first introduced, but this condition is only a transient one and is frequently followed by a sense of comfort rarely experienced when the solutions have a higher temperature. There is some local reaction, of course, but I have never seen any dangerous manifestations. It is much easier to reach the upper colon with such an enema, and this fact alone makes it useful in many cases.

Having discussed the technique of the administration of enemas we will now consider more in detail some of the therapeutic agents to be used.

A considerable variety of drugs from time to time have been recommended, but up to the present time the various salts of quinine have given the most satisfactory results and have been the most constantly and extensively used since their introduction by Lösch in 1875.

If enemas of this drug are properly prepared and administered the outcome in the majority of cases of moderate duration is very satisfactory, and many of the disappointing results following its use are not the fault of the principle but the result of routine and the failure to use it under the most favorable conditions.

There is apparently but little choice between the various salts of quinine, but to be most serviceable the solution should be an acid one. The strength used should vary from 1–1,500 to 1–750, depending somewhat upon the idiosyncracies of the patient. A stronger solution than 1–750 is not required to destroy any amebas which are subject to its action, and besides it is more irritating, and when retained produces unnecessary systemic effects from the absorption of the drug. A weaker one than 1–1,500 does not assure parasiticidal action in a reasonable length of time, and is therefore not to be used.

The fluid used as a vehicle for the application of the quinine also deserves some thought. In 1884 Nothnagel showed that when sodium

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chloride was placed upon the peritoneal coat of the living intestine, active antiperistalsis resulted. In 1894, Grützner injected into the rectum physiologic salt solution in which was suspended finely divided hair, charcoal, and starch. After six hours he demonstrated these substances in the small intestine and stomach. Hemmeter (1902) confirmed these observations, proving that active antiperistalsis followed the introduction of substances suspended in salt solution into the rectum of human subjects, a cat, and a white rat. He further showed that these results were less certain or not to be obtained when solutions of potassium chloride or hydrochloride acid were used. Fasting favored this antiperistalic action and both diarrhea and constipation delayed it; the normal bowel gave the best results. Peristalsis was *not* arrested by this procedure, but continued to control substances well within the lumen of the bowel, while the antiperistalic movements influenced only substances near the walls of the organ.

Accepting these statements as proved, their intelligent application may be of service in the local treatment of amebic dysentery, particularly in the selection of a solvent for use in the application of quinine or whatever other antiparasitic substance is used in enemas.

Not infrequently one of the disagreeable features of the local treatment, whatever the medicine, is the nausea which follows and the consequent interference with digestion and nutrition. One would not think, therefore, of employing a sodium-chloride solution as a vehicle for quinine in such cases, but on the other hand, in certain well-nourished patients with decided intolerance for enemas, this condition may be taken advantage of, and if necessary a sodium-chloride solution enema containing a quarter of a grain of morphine of one-tenth of a grain of cocaine may be administered one or two hours before giving the regular quinine one.

Recent researches have tended to show that the presence of living bacteria are necessary for the propagation of amebas, and if this symbiosis is in reality a necessity to the life of the amebas in the intestine we have an increased incentive to use bactericidal local medication. Bacteria are quite generally considered as having pathologic significance in this disease, partly, no doubt, as a result of their own action and in addition, the symbiosis referred to may result in metabolic changes which may vary with different bacteria and influence the pathologic action both of themselves and of the parasites. Whatever the manner of their action, the clinical importance of limiting the number of intestinal bacteria in this infection is fully recognized. Varous kinds of antiseptic

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irrigations looking to this end have been used and recommended, but in satisfactory concentration they have usually proven either dangerous or impracticable.

In 1902 Freer called attention to the results obtained by Strong in the experimental treatment of eleven cases of amebic dysentery with benzoyl-acetyl peroxide (acetozone). The drug was given both internally and by enema. In these cases the results were encouraging. Of the eleven cases treated, two died, one from a complication of a liver abscess. The other nine at the time of the report were without any symptom of the disease.

The bactericidal properties of this preparation are well known, and its action in acid solution on amebas has been pointed out in Part I of this report. I have never used it alone in treatment, but in acid solution of 1–5,000 to 1–2,000 in combination with quinine 1–1,500 to 1–750, or when used alternately with the quinine enemas, it often gives more satisfactory results than does quinine alone in cases in which it is tolerated.

The inconvenience in securing the solution for private practice and its irritating effects in the colon are the principal objections to it, and the latter is partially overcome when it is used in the cold enemas described above.

Succinic peroxide acid ("alphozone") used in the same manner as acetozone has recently given some encouraging results, and in some very chronic cases the happiest results sometimes follow the occasional substitution of an enema of 1–10 to 1–2 solution of hydrogen peroxide for the quinine one. In a like manner an occasional reaction brought about by an injection of a solution of silver nitrate does good, but the routine use of this drug is not usually satisfactory.

As to the number of enemas to be used, the tendency is to overdo. One enema properly administered is of more value than several given without consideration of the conditions. In the vast majority of cases one to three in the twenty-four hours will be found to be the most satisfactory, and, fortunately, this number fulfills the rational as well as the practical requirements.

Some of the most aggravated cases are those in which a catarrh of the lower bowel has been established by the previous use of enemas too frequently given, of too great a concentration, and which have been introduced into the lower colon in quantities of one liter or less. In not a few cases, owing to the physiological

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action of absorbed quinine or from other causes, it will be found impracticable to give more than one enema daily.

Irritability of the anus and rectum usually may be avoided, but sometimes this condition occurs and it may prove very annoying. After each treatment the external parts should be washed with soap and water and bathed in some antiseptic solution, and when the irritation becomes excessive a cocaine suppository administered just previous to the enema will afford relief, but where possible this is to be avoided, for, in addition to reasons which are obvious, it may interfere with the action of the sphincter to such an extent as to allow the escape of fluid during the operation. Flushing the lower bowel with cold water or some mild, soothing lotion after each treatment gives the most satisfactory results in these cases.

In many of the text-books caution regarding the maximum pressure to be used in the administration of enemas is enjoined in those cases showing advanced ulceration. Theoretically this is good advice, but in actual practice, in the first place, it is impossible to determine the extent of intestinal ulceration in amebic dysentery, and secondly, agreeing with Professor Osler, I must say that the danger from this source is small indeed—so much so that it is doubtful if it deserves consideration in the treatment of a disease where distension of the bowel is so necessary. In over five years' observation in hospital wards, in private practice, and in the morgue, I have never seen evidence of such an accident.

Finally, I wish to emphasize that *no* recommended routine treatment will be found to be satisfactory in all cases of intestinal amebiasis. Each one requires careful study, and variations in the treatment should be made to suit special conditions, and to efficiently do this the physician should either himself administer or be present at the administration of at least the first few enemas given to every patient under his care.

IV. APPENDICIAL AMEBIASIS.

Involvement of the appendix in amebic dysentery, as previously stated, is really a direct continuation of the diseased process from the intestines and therefore, may, be appropriately considered here.

In 100 necropsies on this disease the appendix contained lesions in 14. In 6 the process was an active amebic infection, four times in otherwise apparently healthy appendices, and in one the amebic process was secondary to chronic changes from other causes.

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One was an old chronic appendicitis (nonamebic) associated with a pericecal abscess (amebic) and 7 others were those showing chronic lesions without amebic infection. Four of these seven, however, showed evidence of some acute inflammatory reaction, probably the result of close contact with the amebic process going on in the cecum.

In 4 of the 6 amebic cases death resulted from general peritonitis following perforation, three times in the cecum and once in the descending colon. This entire series was in American soldiers, and necropsies were performed by Dr. Strong and myself, mostly during the year 1900. They were almost entirely chronic cases which gave histories of months of diarrhea, and were in the last stages of the disease when received at the First Reserve Hospital.

In the more recent study of 50 fatal cases, and in many clinical ones, particular attention has been directed to the involvement of the appendix as a complication. However, the statistical results in the fatal cases have in the main been confirmatory of those in the first series.

In every instance where the appendix showed amebic ulceration this has been as a direct continuation of and secondary to a similar process in the cecum. Peritonitis following perforation of a gangrenous amebic ulcer in the appendix occurred in 1 case, but the cecum was also gangrenous. Without lesions amebas have never been found in the appendix.

With these brief data we are better prepared to discuss the clinical side of the problem, which particularly concerns us in this paper. Where the records are complete all cases which have shown lesions of the appendix post-mortem have also given a history of clinical symptoms during life which indicated involvement of that organ. However, on the other hand, *very few of the cases which have manifested clinical symptoms of appendicitis during life have shown lesions of this organ post-mortem.*

The highest mortality in intestinal amebiasis occurs where the cecum is involved, and as appendicitis is found but rarely, if ever, in any other class, it will be seen that post-mortem examinations indicate a much higher percentage with appendicitis than actually exists when all classes of cases are considered. The very great majority of those suffering with amebiasis will not have symptoms

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of appendicitis during life, and this organ will be found healthy at necropsy.

From clinical and post-mortem observations we learn that cases of amebic dysentery with symptoms of appendicitis during life may be divided into three general classes.

(1) Those in which at operation or necropsy the appendix is not found to be diseased.

(2) Those in which at operation or necropsy a chronic appendicitis from other causes and without amebic infection is found.

(3) Those in which at operation or necropsy an amebic infection of the appendix is present.

Clinically examples of the first class are very frequently met with, and sometimes at autopsy no lesions are found which would account for the symptoms during life. However, in one of my cases there was a perececal abscess due to a perforation, and in others there was extensive ulceration of the cecum both with and without adhesions to the omentum and other structures, which accounted for the symptoms during life.

The second class is not infrequently encountered during life, and in the fatal cases there is often congestion, injection of the vessels, and other inflammatory phonemena, due to close relation to the inflamed bowel, but without an amebic process or the presence of the parasites in the appendix.

The third class is the last in order of frequency to be observed in clinical practice, but is more frequent (seven times in the 150 necropsies) in the necropsy records, because, practically, all such cases are fatal.

As previously stated, these seven infections, with one exception, occurred in apparently otherwise healthy appendices, but it is important to remember here that in three of these the cecum was also gangrenous, and death resulted from peritonitis following perforation—twice in the cecum and once in the appendix. In the remaining case the amebic infection was secondary to an appendicitis from other causes.

These statistics show that the majority of appendicial infections are associated with the very severest involvement of the cecum, where death usually results from a perforation in or near the deeply ulcerated and often gangrenous organ, the type of case corresponding so closely to similar ones without infection of the appendix that a differentation during life is often impossible.

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Sometimes when the infection is less intense in the cecum, and the lesions in the appendix more important, the clinical picture of appendicitis is more definite and may with care be diagnosed during life.

The importance of being able to distinguish during life the three classes of cases discussed, from appendicitis due to other causes, as well as to differentiate them from each other is obvious.

As a rule no great difficulty, at least in the Tropics, will be encountered in differentiating the amebic processes located in or about the cecum from appendicitis due to other causes. In the former there is generally a history of dysentery or diarrhea, and a microscopic examination of the stools will usually show amebas, while in the latter a history of previous attacks often may be secured. The general symptoms in the amebic infection are usually less severe and differ to a certain extent from those of the latter. Fever is more commonly slight or even absent; nausea is less frequent (unless the patient is taking enemas), and vomiting is rare. Rigidity of the rectus muscle is nearly always less decided or even absent, and, while pain on deep pressure is quite constant, it is rarely of the very acute type so often seen in other forms of appendicitis. A tumor mass, as in other forms of appendicitis, is usually present, but it more often has a soft, boggy character in the region of the cecum and is rarely circumscribed, definite and low in the right iliac fossa, as in other types of appendicitis. Sometimes, however, where there is a definite appendicitis due to amebas, it may be ushered in as a sharp attack of acute pain, with some muscular rigidity, perhaps a certain degree of fever, an increase in the number of bowel movements, and a leucocytosis of from 10,000 to 20,000.

A differentiation from *each other* of the various types of amebic infections which give the general symptoms of appendicitis during life is a difficult task, and it is even impossible in many instances to say that the appendix is involved at all.

Where the cecum, whether alone or with the appendix, is the seat of dysenteric lesions, pain in the region occupied by these organs is quite a constant symptom, and it may vary from a dull aching one to that of the most acute radiating type. The acuteness of the pain depends, to some extent no doubt, upon the character of the lesions, the stage of the disease, and the general condition of the patient. In well-nourished individuals moderate

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ulceration in or just below the cecum may cause considerably greater pain than similar lesions in chronic emaciated cases. Some of the most deceptive clinical pictures are encountered when the omentum becomes adherent around the cecum, ascending colon, abdominal wall, and liver. When such adhesions form in the region of the appendix considerable thickening occurs and palpation, besides confirming deceptive conclusions, is to be performed with care on account of the danger of rupture.

In the second class of cases, when a chronic adhesive appendicitis has been aggravated by close relation to the diseased cecum, the patient's history usually will show similar attacks antedating the dysentery. The symptoms, which usually come on somewhat slowly, are rarely urgent, and are frequently relieved by a saline cathartic.

We have already seen that in the third class the lesions of the appendix are but a continuation of similar ones in the cecum, and in the rare instances where they are not the clinical picture is the same as that of an appendicitis from other causes.

Treatment.—The majority of cases of amebic dysentery give no symptoms indicating involvement of the appendix during life. No amebas and no lesions are found in this organ post-mortem, and therefore treatment directed specially to the appendix is worse than useless.

When symptoms suggesting appendicitis do arise during the course of the disease the *medical* treatment is that which is applicable to appendicitis from other causes plus high large quinine enemas. Saline cathartics are particularly useful if the patient is in a condition to justify their administration. Thirty grams of Rochelle salts will often relieve, more or less permanently, the symptoms of appendicitis from this cause.

In the early stages of amebic dysentery, in the majority of cases, the appendix could be removed without immediate bad results, but the probable future of the patient must be considered, and this is not best conserved by performing an operation which, for a greater or less time, necessitates a discontinuance of rational treatment of the disease, and which uses up more or less of the strength and energy so necessary for its successful therapeutics. Such precipitate surgery has, in the past, undoubtedly determined the fatal issue in some instances.

We have shown what a variety of pathologic conditions may produce symptoms of appendicitis in intestinal ambiasis and how

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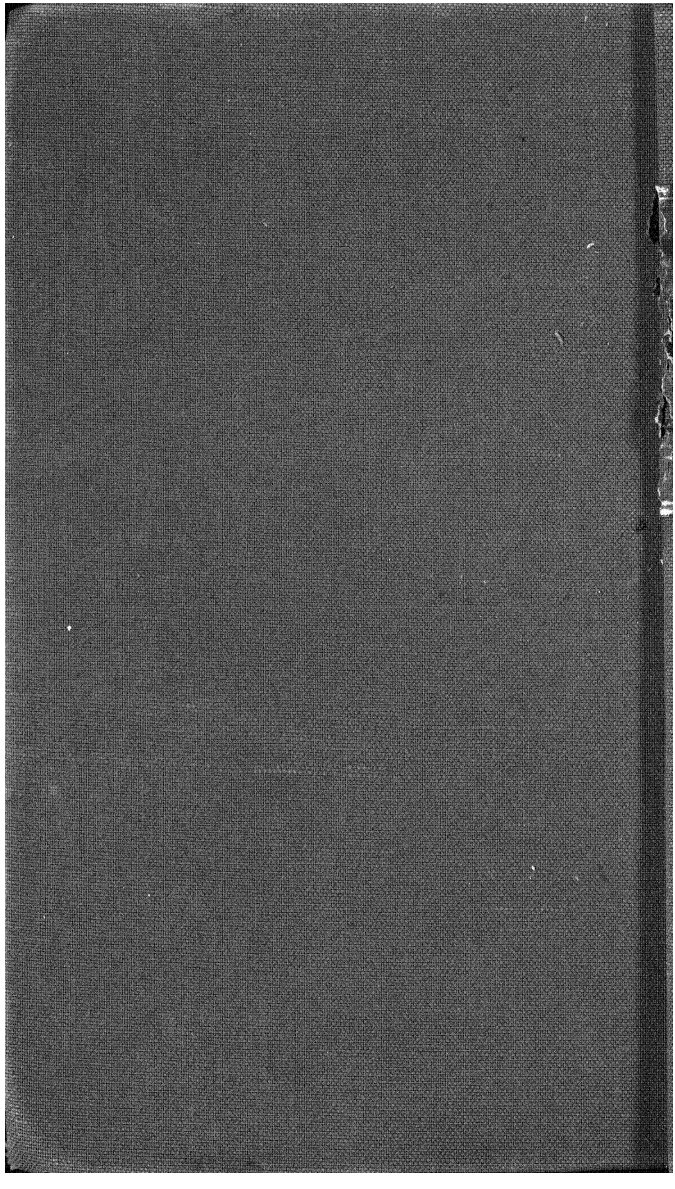

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